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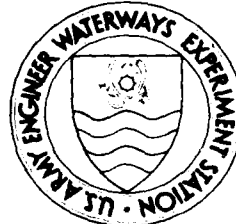**AQUATIC PLANT CONTROL
RESEARCH PROGRAM**

PROCEEDINGS, 21ST ANNUAL MEETING, AQUATIC PLANT CONTROL RESEARCH PROGRAM

17-21 NOVEMBER 1986

MOBILE, ALABAMA

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November 1987

Final Report

Prepared for **DEPARTMENT OF THE ARMY**
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The 21st Annual Meeting of the US Army Corps of Engineers Aquatic Plant Control Research Program was held in Mobile, Alabama, on 17-21 November 1986, to review current research activities and to afford an opportunity for presentation of operational needs. Papers presented at the meeting are included in this report.

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PREFACE

The 21st Annual Meeting of the US Army Corps of Engineers Aquatic Plant Control Program was held in Mobile, Alabama, on 17-21 November 1986. The meeting is required by Engineer Regulation (ER) 1130-2-412, paragraph 4c, and was organized by personnel of the Aquatic Plant Control Research Program (APCRP), Environmental Laboratory (EL), US Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss.

The organizational activities were carried out, and presentations by WES personnel were prepared under the general supervision of Dr. John Harrison, Chief, EL. Mr. J. Lewis Decell was Program Manager, APCRP. Mr. W. N. Rushing, APCRP, was responsible for planning and chairing the meeting. Mr. E. Carl Brown was Technical Monitor for the Office, Chief of Engineers, US Army.

Ms. Jessica S. Ruff of the WES Information Products Division (IPD) edited this report. Ms. Gracie Park of IPD designed and composed the layout.

Director of the WES is COL Dwayne G. Lee, CE. Technical Director is Dr. Robert W. Whalin.

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IN HONOR

This year's proceedings are dedicated to those who, for the sake of science and the pursuit of real-world knowledge, survived the 1986 APCRP field trip to Mobile Bay.

J. L. Decell
PM/APCRP

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21st Annual Meeting
US Army Corps of Engineers
AQUATIC PLANT CONTROL RESEARCH PROGRAM

Mobile, Alabama
17-21 November 1986

MONDAY, 17 NOVEMBER 1986

10:00 a.m. -5:00 p.m.	Registration—Mezzanine
6:30 p.m.	Reception—Mezzanine and Ballroom C

TUESDAY, 18 NOVEMBER 1986
General Session, Ballrooms B & C

8:00 a.m.	Registration Continues—Mezzanine
8:30 a.m.	Call to Order and Announcements —W. N. Rushing, Waterways Experiment Station (WES), Vicksburg, Mississippi
8:35 a.m.	Welcome to Mobile District —LTC Roy Prince, Deputy Commander, USAE Division, Mobile, Alabama
8:45 a.m.	Comments on Integrated Control Technology Development —J. Lewis Decell, Manager, Aquatic Plant Control Research Program (APCRP), WES
9:00 a.m.	Efforts Toward Development of an Aquatic Plant Management Concept for Pat Mayse Lake, Texas —John H. Rodgers, North Texas State University, Denton
9:15 a.m.	Effects of Light Regimes on Tuber Production in Monoecious Hydrilla —Lars W. J. Anderson and David F. Spencer, USDA, Aquatic Weed Control Laboratory, University of California, Davis
9:30 a.m.	Feeding Preference and Stocking Rates of Triploid Grass Carp —Gilbert Pauley, University of Washington, Seattle
9:45 a.m.	Grass Carp Research in the Tennessee Valley Authority —A. Leon Bates, TVA, Muscle Shoals, Alabama
NOTE:	Computer model demonstrations — Admiral Semmes Room, 3rd Floor 9:00 a.m. - 4:00 p.m., 18 Nov 9:00 a.m. - Noon 19 & 20 Nov
10:00 a.m.	BREAK
10:15 a.m.	USAE Division/District Presentations Aquatic Plant Problems/ Operations Activities
12:00 noon	Comments on the afternoon's Working Sessions —J. Lewis Decell, WES
12:15 noon	LUNCH

1:30 p.m.	Working Sessions—Atrium II and
4:30 p.m.	Atrium IV Rooms
3:00 p.m.	Federal Aquatic Plant Management Working Group — Adm. DePineda Room

WEDNESDAY, 19 NOVEMBER 1986
General Session, Ballrooms B & C

Biological Control of Aquatic Plants
—E. A. Theriot, WES, Presiding

8:30 a.m.	Genetic Engineering of Microorganisms —E. A. Theriot, WES
8:45 a.m.	Microbial Control of Hydrilla —S. M. Hennington, WES
9:00 a.m.	Introduction of Insects from Overseas for Hydrilla Control and Feasibility Studies on Manipulating Native Insects for Submersed Plant Control —T. D. Center, USDA, Ft. Lauderdale, Florida
9:15 a.m.	Microbial Control of Eurasian Watermilfoil — Field Studies —H. Gunner, University of Massachusetts, Amherst
9:30 a.m.	Results of Survey for Plant Pathogens on Eurasian Watermilfoil —W. C. Zattau, WES
9:45 a.m.	Literature Search and Preliminary Laboratory Studies on Manipulation of Microflora for Aquatic Plant Control —E. Rejmankova, USDA, Davis, California
10:00 a.m.	BREAK
10:15 a.m.	Literature Search and Preliminary Laboratory Studies on Use of Allelopathy for Aquatic Plant Management —S. Elakovich, University of Southern Mississippi, Hattiesburg
10:30 a.m.	Monitoring Populations of Biocontrol Agents in the Galveston and Sacramento Districts —R. M. Stewart, WES
10:45 a.m.	Introduction of Insects from Overseas for Control of Waterlettuce —Dale Habeck, University of Florida, Gainesville
11:00 a.m.	Influence of Herbicides on Insects Used for Control of Waterhyacinths —A. F. Cofrancesco, WES

Computer-Aided Simulation Procedures for APC
—H. W. West, WES, Presiding

11:15 a.m.	Introduction and Overview —H. W. West
11:30 a.m.	Development and Application of Biological Model (INSECT) for Waterhyacinth Control —J. Wooten and F. Howell, University of Southern Mississippi, Hattiesburg
12:00 noon	LUNCH

1:30 p.m.   Field Trip to Mobile River Delta Area
            Meet in hotel lobby for transportation

THURSDAY, 20 NOVEMBER 1986
General Session, Ballrooms B & C

Chemical Control Technology Development
—H. E. Westerdahl, WES, Presiding

8:15 a.m.   Aquatic Herbicide User Guide

            Herbicide Concentration/Exposure Time Relationships-2,4-D,
                Sonar, Endothall, and Diquat
            —H. E. Westerdahl

8:30 a.m.   Controlled-Release Poly GMA/2,4-D Evaluation
            —R. Gupta, Day Chem, Inc., Dayton, Ohio

8:45 a.m.   Herbicide/Adjuvant and Herbicide Application Technique
                Evaluation for Flowing Water
            —K. G. Getsinger, WES

9:05 a.m.   Field Evaluation of Selected Herbicides for Aquatic Use—Garlon
                and Dichlobenil
            —R. Green, WES

9:20 a.m.   Plant Growth Regulators for Aquatic Plant Control
            —C. Lembi, Purdue University, West Lafayette, Indiana

9:35 a.m.   BREAK

Ecology of Problem Submersed Aquatic Plant Species
—J. Barko, WES, Presiding

9:45 a.m.   Introduction and Overview
            —J. Barko

10:00 a.m. Sediment Chemistry
            —R. L. Chen, WES

10:15 a.m. Diel Water Chemistry
            —G. L. Godschalk, WES

10:30 a.m. Field Studies on the Potomac River
            —V. Carter, USGS, Reston, Virginia

10:45 a.m. Biological Aspects of Studies on the Effects of Aquatic Plants on the
                Environment
            —A. C. Miller, WES

11:00 a.m. Effects of Aquatic Plants on Fish Populations
            —K. J. Killgore, WES

11:15 a.m. Effects of Temperature and Sediment Type on Monoecious and
                Dioecious Hydrilla
            —D. G. McFarland, WES

11:30 a.m. Influence of Environmental Factors on Plant Competition
            —R. R. Twilley, WES

11:45 a.m. Effects of Water Chemistry on Submersed Aquatic Plant Species
            —R. M. Smart, WES

12:00 noon Phenology of Aquatic Plant Species Literature Review
            —G. Pesacreta, Clemson University

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US Army Corps of Engineers
AQUATIC PLANT CONTROL RESEARCH PROGRAM

Mobile, Alabama
17-21 November 1986

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CONVERSION FACTORS, NON-SI TO SI (METRIC) UNITS OF MEASUREMENT

Non-SI units of measurement used in this report can be converted to SI (metric) units as follows:

Multiply	By	To Obtain
acres	4,046.873	square metres
acre-feet	1,233.489	cubic metres
cubic yards	0.7645549	cubic metres
feet	0.3048	metres
gallons per care	0.00093	cubic decimetres per square metre
gallons (US liquid)	3.785412	cubic decimetres
horsepower (550 foot-pounds per second)	745.6999	watts
inches	25.4	millimetres
miles (US statute)	1.609347	kilometres
pounds (force) per square inch	6,894.757	pascals
pounds (mass)	0.000112	kilograms
pounds (mass) per gallon	0.12	kilograms per cubic decimetre
square miles	2.589998	square kilometres
tons (mass) per acre	0.22	kilograms per square metre
tons (2,000-lb, mass)	907.1847	kilograms
yards	0.9144	metres

21st Annual Meeting
US Army Corps of Engineers

AQUATIC PLANT CONTROL
RESEARCH PROGRAM

INTRODUCTION

The Corps of Engineers (CE) Aquatic Plant Control Research Program (APCRP) requires that a meeting be held each year to provide for professional presentation of current research projects and review current operations activities and problems. Subsequent to these presentations, the Civil Works Research and Development Program Review is held. This program review is attended by representatives of the Civil Works and Research Development Directorates of the Office of the Chief of Engineers; the Program Manager, APCRP; and representatives of the operations elements of various Division and District Engineer Offices.

The overall objective of this annual meeting is to thoroughly review Corps aquatic plant control needs and establish priorities for future research, such that identified needs are satisfied in a timely manner.

The technical findings of each research effort conducted under the APCRP are reported to the Manager, APCRP, US Army Engineer Waterways Experiment Station (WES), each year in the form of quarterly progress reports and a final technical report. Each technical report is distributed widely in order to transfer technology to the technical community. Technology transfer to the field operations elements is effected through the conduct of demonstration projects in various District Office problem areas and through publication of Instruction Reports (IR), Engineering Circulars (EC), and Engineering Manuals (EM). Periodically, results are presented through publication of an APCRP Information Exchange Bulletin which is distributed to both the field units and the general community. Public-oriented brochures, movies, and speaking engagements are used to keep the general public informed.

The printed proceedings of the annual meetings and program reviews are intended to provide Corps management with an annual summary to ensure that the research is being focused on the current operational needs nationwide.

The contents of this report include the presentations of the 21st Annual Meeting held in Mobile, Alabama, 17-21 November 1986.

USAE DIVISION/
DISTRICT PRESENTATIONS
AQUATIC PLANT PROBLEMS —
OPERATIONS ACTIVITIES

Lower Mississippi Valley Division
New Orleans District

by
Glen N. Montz*

New Orleans District conducts aquatic growth control under two authorizations: the Removal of Aquatic Growth Project and the Aquatic Plant Control Program. The Aquatic Growth Project is funded 100 percent by the Corps of Engineers and is the original operation and maintenance program for control of unwanted aquatics in Federal navigation projects. The Aquatic Plant Control Program is a cooperative program for the control of aquatics in other public navigable waterways not included in the O&M program. The cooperative program is funded 70 percent by the Corps and 30 percent by the Department of Wildlife and Fisheries in Louisiana. This percent of the cost-sharing program is expected to change to 50/50 soon.

An effective control program was conducted by the Corps of Engineers and the State during FY 86. Control operations were aimed mainly at waterhyacinth, but alligatorweed, watermilfoil, pennywort, water paspalum, salvinia, waterlettuce, najas minor, and hydrilla were also treated. Corps spray crews treated 39,935 acres;** an aerial operation sprayed 1,020 acres; and the state under the work-in-kind and reimbursable treated 38,290 acres.

Field personnel working 10-hr workdays, 4 days a week, have increased productivity greatly over the past several years. This compressed work schedule is used during daylight savings time from April to October each year. Larger airboats and skiffs are being purchased to carry more safely the increased weights of herbicide and gasoline during those 10-hr workdays. Floating aquatics were permitted to grow in two lakes, thus successfully shading and reducing hydrilla infestations. During FY 86 floating and submerged aquatics were treated with 2,4-D, Rodeo, Diquat, Aquathol, and Aquathol K. Since sonar is now available we will purchase some during FY 87 for use on hydrilla. We sprayed Aquathol K on waterlettuce, but the plants were only burned and not completely killed; so, we returned to the use of Diquat on this unwanted aquatic. Aquathol K was unsuccessfully used to treat salvinia, so, we will return to use of Diquat on this aquatic fern which is rapidly increasing in acreage in south-central Louisiana. The Aquatic Growth Control Section is studying the feasibility of purchasing one mechanical harvester in 1990. The use of herbicides will continue, but a shift toward mechanical in the near future is anticipated to supplement our chemical operations.

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* US Army Engineer District, New Orleans; New Orleans, Louisiana.

** A table of factors for converting non-SI units of measurement to SI (metric) units is presented on page xv.

Water samples were collected by Corps personnel and analyzed by a contractor for 2,4-D residue. Samples were collected at various intervals after spraying to correlate time to residue of herbicide. During FY 87 we expect to have water samples analyzed for other herbicides.

The Aquatic Growth Control Section from the New Orleans District attended the Louisiana Pesticide Applicators Association annual meeting in November 1986 for State pesticide applicators certification. All Corps employees were recertified at that time. The Louisiana Legislature recently listed 2,4-D as a restricted-use pesticide.

Communication between aquatic control operations personnel at the District level needs improvement. The success and/or failure of various approaches and techniques should be documented and shared with all interested parties. However, this information does not need to be published. Perhaps a newsletter would be the most appropriate means of communication.

Lower Mississippi Valley Division
Vicksburg District, Lake Ouachita

by
James F. Copeland*

INTRODUCTION

Lake Ouachita is a 48,000-acre lake constructed in 1953 for flood control and hydroelectric power generation. Located 13 miles west of Hot Springs, Ark., the lake receives an annual visitation of 2.2 million.

PLANT PROBLEM

Brazilian elodea, *Egeria densa*, infests the shallow areas of the lake. Because the lake is deep and has steep shorelines, plants grow in bands along the shoreline and shallow coves where light penetration is sufficient for establishment. Over most of the lake the plants cause no problems and provide food and cover to fish and wildlife. However, at beaches, campgrounds, marinas, and boat ramps, stands of Egeria interfere with recreational use.

CONTROL ATTEMPTS

The aquatic plant problem first became noticeable in 1980. Control efforts began in 1983. Rangers in a patrol boat dragged a heavily infested 2-acre swimming area with a hay rake. Because this mechanical means of control was time consuming and removed only about 25% of the plants, it was discarded and new means of control sought.

In June, 1985 an endothall herbicide, Aquathol-K granular, was applied over three 2-acre swimming areas. The application achieved no significant reduction of *Egeria* populations.

Also in 1985, the Arkansas Game and Fish Commission began stocking white amur to control the aquatic plants. They stocked 2,722 white amur of a planned total of 30,000. The Commission has stopped all stocking of white amur because it believes the benefits of *Egeria* to the fish outweigh the inconveniences to swimmers and boaters. The few white amur stocked before the decision have had no noticeable impact.

As part of the white amur stocking project, an attempt was made to map the extent of the weed infestation by flying the lake and taking photographs using an Enviropod camera from the Environmental Protection Agency and infrared film. Unfortunately, after the lake was flown and photographed, the EPA refused

* US Army Engineer District, Vicksburg; Hot Springs, Arkansas.

to develop the film and no other facility could be found to develop the film at an affordable price. Consequently, estimates of the extent of the problem now come from boat surveys.

The Waterways Experiment Station has recommended the application of Diquat dibromide herbicide to the swimming areas. However the logistical problems of guarding treated areas to prevent swimming and fishing for 14 days after applications and the possibility of tort claims may make it impossible to use Diquat.

A winter drawdown has been suggested but not attempted because water levels would have to be drawn down to a point where lake marina operators would suffer. In addition the control would eliminate aquatic plants throughout the lake instead of just the problem areas at beaches, marinas, and boat ramps.

Two other means of control are being considered. Nearby DeGray Lake has had some success in clearing swimming areas by dragging a chain behind a boat. Also, the use of a weed barrier film spread over the bottom to block sunlight to the plants has been proposed but not tried.

North Pacific Division, Seattle District

by
Robert M. Rawson*

The Seattle District has had an operating program since 1980. We have been working with the state of Washington to control Eurasian watermilfoil.

In spite of our efforts, along with those of state, local and private interests, milfoil continues to expand its range rapidly down the Columbia River and into new systems around the state. Control efforts have been hampered by the lack of adequate treatment methods as well as the prolific nature of the plant. We have tried many different treatment methods since we started the program. However, each method has its own set of drawbacks: the cost per acre, effectiveness against the target species, or acceptability to the impacted public.

We have had several significant developments in our program recently. In 1985 legal action was filed by an environmental group in eastern Washington to stop the 2,4-D treatment in Osoyoos Lake. The basis for the law suit was the lack of a worst-case analysis for 2,4-D. We were aware of the possibility of this challenge because the same argument was used against the herbicide treatment of Federal forest lands in Oregon in 1984, resulting in the shut down of spray operations statewide.

We began work on the worst-case analysis in 1985 but did not complete it in time for the treatment season. The analysis was completed in early 1986 so we didn't anticipate further problems. In 1986, however, the Environmental Protection Agency denied our Section 18 permit, and the chemical portion of our treatment program was again cancelled.

Because of these problems, the Washington State Department of Ecology, our state sponsor, has decided to pursue mechanical options for the immediate future. Mechanical harvesting is still being done in the Seattle area and rotovating has been initiated in Osoyoos Lake and the Pend Oreille River. The rotovator being used is a new design and seems to be much more promising than the old equipment.

We are currently in the process of supplementing our authorizing documents to expand the use of rotovating, expand the use of the herbicide endothall, and to add the herbicide fluridone to the program.

We believe that the use of 2,4-D in our program is over until EPA completes its reregistration process. If anyone needs a copy of our worst-case analysis, please let me know. It is quite extensive and provides a lot of good information. It was prepared by the environmental consulting firm of Tetra-Tech.

We hope that an effective biological control agent can be developed and believe that research in this area should be a very high priority for the Corps.

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* US Army Engineer District, Seattle; Seattle, Washington.

South Atlantic Division, Charleston District

by
John L. Carothers*

Aquatic plants infest public waters throughout South Carolina, but most of our worst problems occur in the coastal plain. The most troublesome species include elodea, hydrilla, water primrose, and alligatorweed. Hydrilla continues to spread.

The aquatic plant control program in the Charleston District is accomplished under a cooperative agreement with the S. C. Water Resources Commission. The Commission subcontracts fieldwork to other state agencies and commercial applicators. To date, no fieldwork has been done on any Federal water resource project.

During the 1986 growing season, we treated 4,440 acres with herbicides including Diquat, Endothal, Rodeo, and 2,4-D. Grass carp were stocked in six lakes having a total surface area of about 622 acres.

Expenditures were as follows:

Aquatic plant surveys	$ 30,000
Herbicide application	660,000
Grass carp and fish barriers	30,000
Program management by the WRC	50,000

The cost of fieldwork was $770,000 of which the State share was $231,000 and the Federal share was $539,000. District expenditures for planning and contract administration were $58,000, bringing the total cost of the FY 86 program to $828,000.

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* US Army Engineer District, Charleston; Charleston, South Carolina.

South Atlantic Division, Mobile District

by
Michael J. Eubanks*

Aquatic plant management activities in the Mobile District during 1986 continue to be concentrated in four geographical areas: the Mobile Delta, Coffeeville Lake, Lake Seminole, and the Tennessee-Tombigbee Waterway (TTW).

The State of Alabama Department of Conservation and Natural Resources, under a Cooperative Agreement with the Mobile District (signed on 28 January 1986), conducts the activities in the Mobile Delta and Coffeeville Lake. Mr. Joe Zolczynski, along with that agency, will summarize the work carried out under the Cooperative Agreement for Eurasian watermilfoil, hydrilla, and other problem species.

Lake Seminole continues to contain the heaviest infestation of aquatic weeds of all the Corps reservoirs. Major weed species include hydrilla, giant cutgrass, Eurasian watermilfoil, waterhyacinth, and alligatorweed. Major aquatic plant control activities this year involved:

Hydrilla: 171 acres (Aquathol-k) in boat channels on 24 July
275 acres (SONR-SRP) on 16 October

Giant Cutgrass: 550 acres (Rodeo)

Waterhyacinth: 300 acres (2,4-D, DMA) 16-17 April
520 acres (2,4-D, DMA) 7-8 October

For hydrilla treatment next spring, we have ordered 33,000 lb of SONAR-SRP.

Aquatic plant management on the TTW was restricted to monitoring for possible introduction of exotic species. The species composition consists predominantly of beneficial native species, but a significant reduction in acreage of aquatic plants was noted in all of the TTW lakes during 1986.

Future research areas that would assist our programs include:

Aquatic

a. Definition of "problem" aquatic plants.

b. Herbicides or water poison issue.

c. Innovative financing to assist local sponsors in meeting 50 percent cost-sharing requirement contained in the new Water Resources Development Act of 1986.

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* US Army Engineer District, Mobile; Mobile, Alabama.

South Atlantic Division, Jacksonville District

by
John P. (Pete) Milam*

INTRODUCTION

The Jacksonville District conducts aquatic plant control in Florida under two authorizations: the River and Harbor Act of 1899 and Public Law 89-298.

- The River and Harbor Act of 1899, known as the Removal of Aquatic Growth Project (RAGP), provides for the control of aquatic weeds in Federally authorized navigation projects which include the St. Johns River (maintained by Corps of Engineers (CE)), Crystal River, Withlacoochee River, Kissimmee River, Okeechobee Waterway (Caloosahatee River and St. Lucie Canal maintained by DNR). This program is funded 100 percent by the Corps under the O&M General account.

- Public Law (PL) 89-298, known as the Aquatic Plant Control Program (APCP), provides for control of all nuisance aquatic plant species in public water in Florida for the purpose of navigation, flood control, agriculture, public health, recreation, or other purposes that may have major economic significance within the State. The APCP currently includes 266 public water bodies in the state. The APCP is a cooperative program and the costs are shared by the CE (70%) and State of Florida (30%). The program is funded by the CE construction general budget and has an annual Federal funding ceiling of $12 million.

OPERATIONS

The CE has been involved in aquatic plant control in Florida since the passage of the River and Harbor Act of 1899. The Corps did not begin its Cooperative (70-30) aquatic plant activities in Florida until 1958 with the passage of PL 85-500. Since 1958 there have been numerous changes and advances made in our Aquatic Plant Control Program. The most recent and significant change has been the establishment of a Cooperative Agreement between the CE and the State of Florida for control of aquatic plants. This Cooperative Agreement was instituted in Fiscal Year 1986 and has eliminated the cumbersome methods by which our Program had to be administered. Under the Cooperative Agreement, the majority of the actual control operations have been assigned to the Florida Department of Natural Resources (DNR). DNR has in turn subcontracted their assigned work between five State water management districts and seven counties. The Jacksonville District's in-house crews working out of the Palatka Natural Resource Project Office are responsible for control operations in the St. Johns River between

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* US Army Engineer District, Jacksonville; Jacksonville, Florida.

Jacksonville, Lake Washington, and a portion of the Oklawaha River including Lake Oklawaha. Tables 1 and 2 provide a breakdown of the aquatic plant control efforts in Florida for FY 1986.

Table 1
Acres Treated in Florida
Fiscal Year 1986

Floating plants (waterhyacinths, waterlettuce, hyacinth/lettuce mix)	29,556
Hydrilla	9,468
Minor plants	2,226
Total	41,250

Table 2
Expenditures for Operations in Florida
Fiscal Year 1986

Agency	APC	RAG	Total APC/RAG
CE	$53,900	$755,895	$809,795
Fla. DNR	$2,991,850	$1,471,063	$4,462,913
Total	$3,045,750	$2,226,958	$5,272,708

AQUATIC PLANT MANAGEMENT PROBLEMS 1986

Waterhyacinth control in Lake Okeechobee was the principal concern in the Jacksonville District this year. Due to a large phosphorus dependent blue-green algae bloom that developed on Lake Okeechobee in August, Florida banned all herbicide treatment of aquatic plants in Lake Okeechobee for a period of 30 days. This 30-day ban coupled with increasing water levels that were releasing more plants into the open water and the already overabundance of waterhyacinth and waterlettuce in the lake at the time of the ban caused us great concern in our ability to maintain navigation in the Okeechobee Waterway. During this period, a cable barrier had to be placed across an adjacent canal north of the Okeechobee Waterway navigation channel in order to prevent large jams of waterhyacinth from entering into the waterway. An anticipated 3 months is needed to gain maintenance-level control of waterhyacinth in Lake Okeechobee.

Hydrilla continues its prolific growth throughout most of Florida. We anticipate a substantial increase in hydrilla treatment in the following years now that fluridone has been labeled by EPA. Our use of fluridone while under the experimental label has given us encouraging results. Thus we are looking forward to being able to add this new "tool" in our management plan in FY 1987.

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South Atlantic Division
Jacksonville District, Puerto Rico

by
John P. (Pete) Milam*

INTRODUCTION

Aquatic plant management operations in Puerto Rico are directed primarily towards waterhyacinths. The program became operational in 1982, after being in the planning stage since 1974 pending the resolution of several environmental issues. The local interests, the Department of Natural Resources, have agreed to hold and save the United States free from claims that may occur from control operations and to share 30% of the costs of the operations. Aquatic plant control in Puerto Rico is conducted under the authority of Section 302 of Public Law 89,298, known as the Aquatic Plant Control Program.

OPERATIONS

The Environmental Quality Board (EQB) of Puerto Rico has been concerned about potential effects 2,4-D may have on the environment and whether or not water quality standards were being met. In order to determine the effects 2,4-D has had on the environment, DNR treated a portion of Rio De La Plata to monitor 2,4-D residues and various environment parameters. The results of the monitoring indicated that the treatment had a negligible effect on the environment and did not violate the water quality standards established by the EQB. However, some of the samples taken after treatment showed elevated phenol and nutrient levels.

Based on the results of the monitoring, EQB approved the use of 2,4-D in certain canals and streams. Phenol and nutrient monitoring continued in conjunction with spray operations in Cano Cabo Caribe during FY-86.

DNR has achieved maintenance control of waterhyacinths in most of the canals now being treated. However, in some of the canals, aquatic grasses are now encroaching on the areas once occupied by waterhyacinths. The Corps and DNR have been working with EQB during the past year to get RODEO approved for use. The EQB approved its use in May 1985 for non-aerial applications. DNR's subsequent use of RODEO has been successful.

During FY-1986, control operations totaled $220,413.27. The local share was $66,123.99. Reimbursement to the Commonwealth by the Corps was $154,289.28.

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* US Army Engineer District, Jacksonville; Jacksonville, Florida.

SUMMARY

In summary, floating vegetation is under maintenance control throughout most of Florida. Hydrilla continues to make its spread with new infestations continuously being found. This plant is still regarded as a serious threat to navigation in the State and sustained efforts of control remain a high priority with the Jacksonville District. Control operations in Puerto Rico are progressing slowly due to environmental issues; at this time most of the canal sites are under maintenance control.

South Atlantic Division, Wilmington District

by
Charles R. Wilson*

Aquatic Plant Control in North Carolina is governed by the North Carolina Interagency Council on Aquatic Weed Control, a council composed of representatives from State and Federal resource agencies, conservation groups, private industry, and local universities. The Wilmington District and the North Carolina Department of Natural Resources and Community Development are the lead agencies charged with the coordination and funding of control operations. Treatment species presently under the North Carolina Aquatic Plant Control (APC) Program are alligatorweed and hydrilla.

Hydrilla was identified at 2 new sites in 1986, bringing the total number of known sites in North Carolina to 20. All of these sites have been considered for inclusion under the North Carolina APC Program. Seven sites have been approved for treatment, and three are under investigation.

Growth of hydrilla in Fred Bond Park Lake was not excessive this year, and no weed control operations were implemented. We will continue to monitor this site, and treatment will be made if needed.

Three of the approved sites are water supply and before FY 86 had not been treated due to a reluctance by health agencies within the State to use herbicides in water supply reservoirs. The introduction of triploid grass carp into North Carolina waters was approved by the North Carolina Wildlife Resources Commission in 1985. In October of that year, sterile triploid grass carp were stocked in Lake Wheeler, at a rate of 20 fish per vegetated acre. These fish provided good control early in the recreation season, but by mid-summer hydrilla reached the problem level. Transects surveyed prior to carp stocking were resurveyed in September of this year. Comparison of fathometer tracings of pre- and post-stocking surveys showed no significant reduction in hydrilla along the transects; however, signs of grass carp feeding were evident in other areas. The grass carp weigh 2 to 3 pounds now, and it is expected that they will be large enough during the 1987 growing season to provide control throughout the year. The use of grass carp at the other potable water reservoirs will be considered if good results are obtained at Lake Wheeler.

During FY 86, 32 acres of hydrilla at three lakes in William B. Umstead State Park were treated with granular Sonar. Control was complete and persisted throughout the recreation season. While no hydrilla is known to be growing in any of the Umstead Park lakes at this time, tubers are present and regrowth is expected. Thus it appears that all three lakes in Umstead State Park will require herbicide treatment in FY 87.

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* US Army Engineer District, Wilmington; Wilmington, North Carolina.

The range of alligatorweed in North Carolina extends from the northern to the southern State line and from the coast to the piedmont. Four thousand acres of alligatorweed from 16 counties in North Carolina have been identified.

Alligatorweed control under the North Carolina APC Program for FY 86 included the application of Rodeo to problem areas in the Scuppernong River, the Little River, and Pasquotank River basins. Successful FY 85 alligatorweed control efforts reduced the area which required treatment on the Scuppernong and navigable tributaries to 3 acres. Herbicide application was expanded in the Scuppernong basin this year to include major drainage canals with an additional 11 acres treated in those areas. Alligatorweed in the Little River basin required only limited spot application. The Pasquotank River basin was added as a treatment area this year with 27 acres treated.

Alligatorweed is under maintenance control in the Scuppernong River and Little River and their major tributaries; consequently, these sites would require only spot treatments in the future. The Pasquotank River and tributaries will require extensive weed control operations in 1987; however, we expect to bring the Pasquotank under maintenance control by 1989. Rodeo provided good control in the lower, permanently flooded reaches of the Scuppernong Canals. Upper reaches which are intermittently flooded were not treated because we have no suitable herbicide for those areas. Since Rodeo has been found to be ineffective for control of alligatorweed in this situation, we had intended to use Arsenal, a ditch bank herbicide, in the canals while they were dry. However, comments from the Environmental Protection Agency during review of our Supplemental Information Report indicated that the use of Arsenal in these areas was inappropriate. We are concerned that if we cannot find an effective herbicide to control alligatorweed in the upstream portion of treatment canals, they will continue to reinfest sites downstream.

The North Carolina APC Program continues to grow. In fact, planning efforts during FY 86 included the completion of supplemental information reports for the inclusion of major drainage canals and sites in the Pasquotank River basin the program, as well as ongoing studies for the inclusion of new treatment species and problem areas.

Studies for the inclusion of *Egeria sp.* as a new problem plant species are ongoing. Three problem areas have been identified with potential for inclusion in the North Carolina APC Program: Lake Reidsville, Oak Hollow Lake, and Lake Gaston. All of these sites are important recreation lakes. Also Lake Reidsville and Oak Hollow Lake serve as water supply reservoirs. Lake Gaston on the North Carolina/Virginia boundary may qualify for inclusion in the program; however, Gaston's present aquatic weed problems occur in Virginia waters, and the Wilmington District has no Memorandum of Agreement with Virginia for weed control. We are currently working with the Commonwealth of Virginia and Virginia Power Company to develop a cooperative weed control program for Lake Gaston.

We have recently initiated investigations of the Lumber River as an alligatorweed treatment area. The Lumber River is being considered by North

Carolina for Wild and Scenic River designation and is an important recreation corridor for canoeing, boating, and fishing. Local residents report that the infestation has occurred within the last 2 to 3 years. Since Alligatorweed covers from about 50 to 90 percent of the river surface area along infested reaches, total river blockage is common. Present estimates indicate about 31 acres of alligatorweed on the mainstream of the river over a 16.5 mile study area.

Two new hydrilla sites, Lake Butner and Lake Creedmore, are being considered for inclusion in the program. Both public fishing lakes and water supply reservoirs, these sites are of particular concern to the Wilmington District since they are located on two major tributaries to Falls Lake and could be a source for hydrilla infestation there. Falls Lake is a Corps of Engineers' multipurpose reservoir with about 12,000 surface acres. Over one-half of the reservoir is less than 10 feet deep and has potential for hydrilla infestation.

The District provided support to the North Carolina Department of Agriculture in a project to develop a cold tolerant flea beetle. Initial releases of selected cold tolerant stocks were made in southern North Carolina in September of 1985. These sites were inspected in the spring and summer of 1986, but no feeding damage or flea beetles were found thus it was concluded that the flea beetles did not overwinter.

No aquatic plant control is presently being undertaken at any Wilmington District reservoirs; however, several of our reservoirs are in close proximity to infested lakes. Our primary efforts at this time have been toward educating the general public and reservoir management staff. Also project biologists have been trained to recognize potential problem plants, and all boat ramps have been posted with signs instructing users to clean their boats and trailers prior to launching.

The extent of water primrose growth in Falls Lake was about the same this year as it was last summer. So far, boat channels remain open, and the weed is not significantly interfering with recreation or project operation. No treatment is planned at this time; however, if water primrose begins to cause problems, a control program will be developed.

For this fiscal year and in the future, we plan a continuation of the existing control program for hydrilla and alligatorweed with expansion to cover new treatment species and sites as needed.

Southwestern Division, Fort Worth District

by
Ed Moyer*

The Fort Worth District has a 5-year open-season aquatic plant control contract, based on a 70-30 cost-sharing agreement with the Texas Parks and Wildlife Department, whereby any of the 21 project managers can join the contract program in any year within the 5-year span. The contract will be renewable on an every consecutive 5-year period. Sam Rayburn Reservoir and B. A. Steinhagen Lake have been in the cost-sharing program for many years now, with treatment at the maintenance level for waterhyacinth and alligatorweed. In the past, there have been annual contracts, 2-year contracts, etc.; with a 5-year contract, there is little to change administratively. The one project that we did pick up as a newcomer to the cost-sharing contract this 1986 recreational period (the upcoming 1987 recreational year spraying will represent the third year of the existing 5-year contract) Lake O' the Pines. Water lillies and some hyacinths were sprayed with good results, but their biggest program is with the Elodea spp. growing in exuberance. The long growing season, with nutrients in the water, and minimal winters the last few years have produced some significant growth. In response to the need, the project management produced O&M monies to attempt a control of Elodea spp. This was the first use of the herbicide product Sonar in the Fort Worth District reservoirs, the product only recently being registered for use within the State.

The results of the treatment were not good, even with a retreatment of the major areas by the Texas Parks and Wildlife Department. It is possible the treatment got under way too late to be effective (being later than an early spring application), a point that we will try to remedy this upcoming year. However, the Elodea spp. have really taken over rather suddenly, even with some lower than normal winter exposures there. This type plant also seems to have gathered strength in numbers in Sam Rayburn Reservoir. It would appear that reservoirs in East Texas will continue to have a problem with Elodea spp. for some time unless significant natural weather occurrences help kill back portions of the biomass.

Project managers are presented with a few alternatives to combat this increase of Elodea spp. With the current cost of treatment of 1 acre using Sonar or its equivalent, they are looking at a $400-plus figure per acre for treatment of water less than 6 ft depth. Depths greater than 6 ft would require a double rate with double cost. It is inconceivable that O&M monies will be enough to control major areas of growth. The only realistic alternative is to continue with a maintenance program at priority areas only, mainly those sites where navigation will be

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* US Army Engineer District, Fort Worth; Fort Worth, Texas.

significantly disrupted. Opening up lanes to major marina areas would also have to be considered. It is possible that, in the future years, there will be requests for relocation by some marina operators, or owners pulling up their operation in especially dense areas.

Needless to say, most bass fishermen are not overly concerned with this problem. Other than the East Texas reservoirs noted above, there are not major aquatic weed problems at other reservoirs at this time.

Southwestern Division, Galveston District

by
Joyce Johnson*

Although the Galveston District boundaries extend generally from the Texas Gulf coast to about 200 miles inland, the District is responsible for the Aquatic Plant Control Program operations in the entire State of Texas. Fort Worth District is responsible for aquatic plant control operations in project areas, such as Sam Rayburn Reservoir and Dam B within the boundaries of their district, and Tulsa District manages Pat Mayse Reservoir, which is also in the eastern part of Texas. The control of noxious aquatic plants has been managed by the Galveston District since 1968. A General Design Memorandum and Environmental Statement for the control of alligatorweed and waterhyacinth were published in the early 1970's. In November 1985, a supplement to the GDM for the control of submersed species was approved. Most of the waterhyacinth fieldwork is within the District boundaries. However, hydrilla has spread north within the state, particularly in east Texas. In April 1986, the first treatment of hydrilla was carried out as part of the Federal program. For this first, very successful treatment, fluridone was used in a 1,600-acre potable water supply where a 300-acre of infestation was severely limiting access. It is anticipated that treatment of hydrilla will be expanded during the spring of 1987.

During the 18-year history of the Aquatic Plant Control Program in Texas, The Galveston District has had four cost-sharing contracts with the State of Texas. The Texas Parks and Wildlife Department plays an active, vital role in the Texas program, performing most of the fieldwork and all of the herbicide spraying. In the past, 2,4-D (dimethylamine salt of 2,4-dichlorophenoxyacetic acid) has been used exclusively in the herbicide program; however, as I mentioned, fluridone is being used in the effort to control hydrilla. Alligatorweed is controlled by *Agasicles* flea beetles in Texas at the present time. Populations that dwindled during the late 1970's have been supplemented by releases during the past 5 years by the Waterways Experiment Station (WES) with insects provided by Florida or collected in Louisiana. In addition, WES has introduced other biological agents including *Neochetina bruchi*, *Neochetina eichhorniae*, and *Sameodes albiguttalis* for the control of waterhyacinth and *Vogtia malloi* for alligatorweed control. The State of Texas is continuing the work started by WES by incorporating the biological agents in the cost-sharing program. This past year, state crew leaders have met with the WES team to learn the technical aspects of releasing insects, and have taken trips to J. D. Murphree Wildlife Management Area to collect the insects. Several nursery areas for these species have been established during the

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* US Army Engineer District, Galveston; Galveston, Texas.

WES effort. The acceptance by the Texas Parks and Wildlife Department of a renewed emphasis on biological control of aquatic species has been due, in large part, to the quality of the work and support from the WES team headed by Ed Theriot. The early releases of a few individual insects by Al Cofrancecso and Mike Stewart has come a long way in Texas.

During the spring-summer herbicide spray program, the Texas Parks and Wildlife Department had 5 crews stationed in three areas treating waterhyacinth. Through September, about 18,000 acres have been treated both by boat and aerial spraying. After 2 years of harsh winters, and a severe drought in the south-central portion of Texas, waterhyacinths infestations have increased tremendously this past growing season.

It is expected that with the additional treatment of submerged species, primarily hydrilla, the program will continue to expand in the next few years. Although proposed control of hydrilla is limited to treatment of boat ramps and access in 11 presently infested lakes in Texas, several additional infestations have been identified and studies to include those areas will be initiated during 1987. The herbicide costs involved in treatment of hydrilla are so much greater than costs associated with treatment of waterhyacinth; the program will cost nearly three times more than in the past. It is clear, then, that a less costly, environmentally compatible method of hydrilla control is needed in the Galveston District program. Again, the District plans to utilize the expertise of the WES Aquatic Plant Control Research Program.

During 1987, the Galveston District will negotiate a cost-sharing cooperative agreement with the State of Texas. During the past year, the State has begun an automated program to simplify the program billing and to reduce the administrative costs. Another goal of the District program is to continue the communication between the Galveston District and the Operation Support Center in the Jacksonville District to incorporate their extensive experience in solving operational problems.

Southwestern Division, Tulsa District

by
Loren M. Mason*

INTRODUCTION

Pat Mayse Lake, Texas, is the only Tulsa District lake experiencing an aquatic plant infestation problem to date. Eurasian watermilfoil is believed to have been introduced into Pat Mayse Lake in 1976 but did not become a problem until June 1978 when approximately 74 acres of the lake became heavily infested, eliminating many water recreation activities. By fall 1981, over 1,000 acres had become infested. On June 14, 1983, the Tulsa District chemically treated 90 acres of the milfoil with aquathol K (dipotassium salt of endothall, a granular aquatic herbicide). Virtually 100-percent control was achieved in the treated areas with no adverse effects on water quality or nontarget species. The 1983 control program was initiated in support of opening up recreational swimming beaches and boat launching ramps. Since the 1983 treatment program, milfoil has continued to interfere with recreational activities. To solve the ongoing aquatic problem, the Tulsa District initiated an experimental mechanical harvesting activity in selected areas of Pat Mayse Lake during 1985 and 1986. (See Figures 1 and 2 for acres infested.) As a result of the water and soil sediment studies conducted by North Texas State University since 1981 and experience in both chemical and mechanical control, it was concluded that an operations manual was needed to provide guidance and proposed actions related to forecasting aquatic plant growths at Pat Mayse Lake, and to identify resources required to respond to future problems and control programs.

OPERATIONS CONTROL MANUAL

Actions were begun in May 1986 to develop an Operational Control Manual as a tool in assisting the decision-making process associated with available strategies and options for control. Alternatives are considered and addressed in a comprehensive yet understandable manner. Instructions for determining and making cost calculations are included along with specific guidance that links the frequency of occurrence and magnitude of the anticipated problem along with an appropriate monitoring program. The manual also provides guidance regarding natural "triggers and timing of management efforts to allow sufficient response for budgeting and required coordination with state and Federal agencies. The manual was developed around three major operational principles (Figure 3) and seven key steps in support of data development and decision making (Figure 4).

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* US Army Engineer District, Tulsa; Tulsa, District.
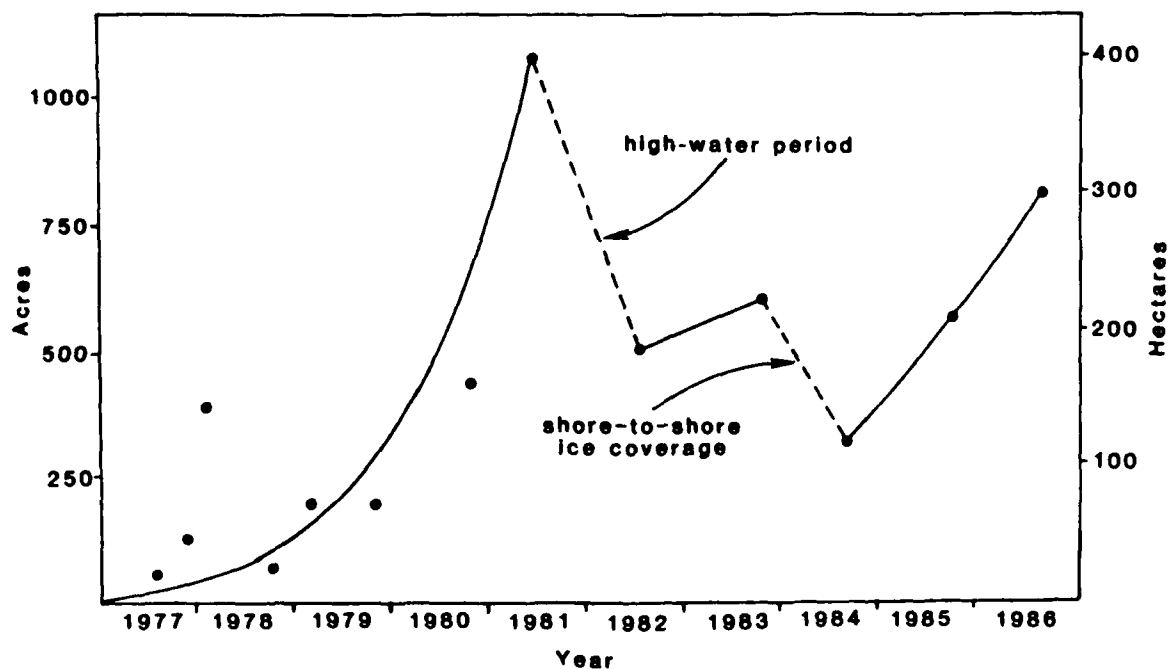
Figure 1. Area covered by *Myriophyllum spicatum* L. in Pat Mayse Lake versus time
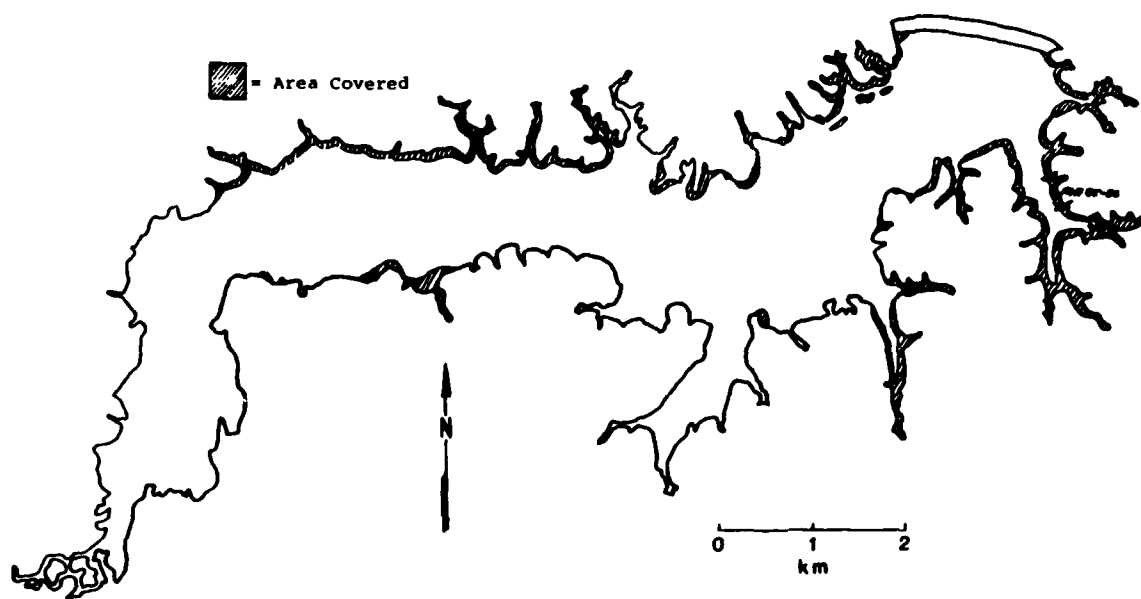
Figure 2. Pat Mayse Lake, area covered by *Myriophyllum spicatum* L. in August 1986 (MH = site of mechanical harvesting treatment area, 1985 and 1986)

1. **AQUATIC VEGETATION MANAGEMENT** with emphasis on *M. spicatum* (Eurasian watermilfoil).

2. **WATER QUALITY MANAGEMENT** with emphasis on designated usages.

3. **SEDIMENTATION AND USEFUL RESERVOIR LIFE** with emphasis on flood control, sedimentation rates, recreation impacts, and reservoir volume.

Figure 3. Operational decisions, Pat Mayse Lake

1. Identification and definition of a problem requiring attention.

2. Anticipation of a developing problem.

3. Review of the range of available solutions (tactics).

4. Choice of a tactic or tactics and integration into long-term management strategies (in terms of anticipated and desired results).

5. Application of a tactic.

6. Monitoring and evaluation of results from tactic application.

7. Feedback of results and integration into long-term management strategies.

Figure 4. Steps in support of operational decisions, Pat Mayse Lake

As developed, the manual addresses operational management problems that are site specific to Pat Mayse Lake with emphasis on *Myriophyllum spicatum* L. and its impact upon designated usages such as drinking water supply, wildlife enhancement, recreation, sedimentation, useful reservoir life, flood control, and recreation impacts that have been studied over the past 5 years at Pat Mayse Lake. However, the manual, with minor modification and site-specific data, can be applied to other reservoirs within the Tulsa District and other Districts with similar problems.

CONCLUSION

It is anticipated that the Operational Control Manual will undergo many changes and revisions over the next several years. The Tulsa District recognizes that the manual will not provide instant and comprehensive guidance in all situations due to the multicomplex factors associated with an aquatic plant control program. It is, however, the District's intent to improve operational forecasting capabilities and response actions in planning and implementing an aquatic plant control program. It is, therefore, with these acknowledgements that the Operational Control Manual is available for review and use by other Corps elements as is appropriate.

ACKNOWLEDGMENTS

The author wishes to acknowledge the exceptional work performed by Dr. John H. Rodgers, Jr., and graduate students under his supervision from NTSU over the past 5 years.

Aquatic Plant Control Operations Support Center

by
Michael Dupes*

The Aquatic Plant Control Operations Support Center (APCOSC) was formally established in FY 81. The Center is located within the National Resource Management Section, Construction-Operations Division, of the Jacksonville District.

CENTER RESPONSIBILITIES

The policies, functions, and procedures for the use of Center services are set forth in Engineer Regulation 1130-2-412. The regulation describes the relationship between the APCOSC, the Office, Chief of Engineers (OCE), and the U.S. Army Engineer Waterways Experiment Station (WES) establishes the following functions of the Center:

- Provide technical guidance to Corps Districts in planning phases of aquatic plant control program.
- Provide technical guidance to Corps Districts in the operational phases of aquatic plant control programs.
- Provide technical expertise and/or operational personnel and/or equipment to respond to localized short-term critical situations created by excessive growths of aquatic plants.
- Provide assistance to OCE for the training and certification of Corps application personnel.
- Upon request, assist WES in field application and evaluation of newly-developed control techniques or procedures.
- Provide assistance to OCE in the development of a comprehensive, Corps-wide aquatic plant control program.

FISCAL YEAR 1986 ACTIVITIES

Table 1 provides a listing of the services performed by the Center and the types of users to which these services were provided. During FY-86 a total of 80 requests for assistance were received and responded to by the APCOSC. Since established, the Center has responded to a total of 715 requests.

Corps Districts accounted for 31 or 38.7 percent of the total services during the year. The next most frequent user was Corps Divisions with 15 requests or 18.8 percent. State and local governments were the third most frequent user with 14 requests or 17.5 percent of the total. Planning and operational assistance were the most frequently requested services at 40 and 49 percent, respectively.

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* US Army Engineer District, Jacksonville; Jacksonville, Florida.

Type Assistance	Corps				Other Federal	Other Country	State Local	Industry	Private	Total
	OCE	WES	Div.	Dist.						
Planning	1	2	10	13	1	0	5	0	0	32
Operations	0	1	5	14	7	0	9	1	2	39
Research	0	2	0	0	0	0	0	0	1	3
Training	0	0	0	4	1	0	0	1	0	6
Totals	1	5	15	31	9	0	14	2	3	80

PLANNING

There were 32 planning services performed during the year ranging from simple explanations of the Aquatic Plant Control Program planning process to detailed assistance in the formulation of planning documents. Valuable assistance was given to the Savannah District in developing a cooperative agreement with the State of Georgia.

OPERATIONS

Operationally-oriented services accounted for the largest portion of the work this year. The Center continued its program to collect and ship insect control agents to other states to control alligatorweed. A total of 53,200 flea beetles were collected and shipped to 10 separate agencies or projects for operational release.

Operational assistance was given to the Wilmington District to chemically control alligatorweed in Pasquotank and Scuppernong Rivers. The Center also provided an airboat and operator from the Palatka Resource Office for the control operations.

The remainder of the operational services consisted of providing information or evaluations on the potential use of specific chemical, biological, or mechanical methods for control.

RESEARCH

The Center's authority and expertise do not include conducting research. However, we are responsible for providing other research organizations with operational data or information to assist with research. This year, for example, personnel from our Palatka Project Office assisted WES in the layout application and evaluation of experimental herbicides in Lake Ocklawaha.

Palatka personnel also assisted WES and University of Massachusetts researchers by designing and building a special pump and application system to apply experimental Biological Control agents to Eurasian Watermilfoil. Onsite assistance was also provided to the researchers in the use of the equipment.

TRAINING

This year's training assistance consisted primarily of providing materials or making referrals to others for training purposes. Significant effort was given, however, in writing portions of the herbicide manual being developed by WES and in developing a slide presentation on Aquatic Plant Control for the Armed Forces Pest Management Board. This presentation will be used for certification training of Navy personnel.

STATUS OF THE APCOSC

The Center was able to complete most service requests during the fiscal year in an adequate and timely manner. However, the Center was handicapped by the loss of experienced personnel. This caused some delay in responding to requests, especially those requests that involved extensive use of manpower.

Alabama Department of Conservation and Natural Resources

by
Joe Zolczynski*

The Alabama Department of Conservation and Natural Resources, Game and Fish Division, completed the following work on aquatic plant management in the public waters of Alabama during 1986.

Eurasian watermilfoil (305 acres) was chemically treated in the Mobile Delta, and fishing area, and boat trails were opened in Big Batteau, Chocalata, Chuckfee, Bay Minette Bay, Bay Minette Basin, Bay John, Polecat Bay, and Grand Bay. In these treatments, 1,525 gallons of 2,4-D amine were used. Surveys indicated that the Eurasian watermilfoil acreage decreased and that native plants were growing successfully in chemically treated areas.

Hydrilla in Coffeeville Reservoir was chemically treated using endothall compounds. Although very little biomass of the plant remains present, surveys indicate a light coverage of approximately 15 acres. Twenty-six acres were treated three times with surveys being conducted twice each month during the growing season. The biomass of hydrilla was therefore, kept to a minimum. This should be effective in reducing its spreading to other waterways.

Reports of hydrilla were checked, and no new infestations were found. Also, surveys indicated a complete absence of water hyacinths from the delta. Indications are that the hyacinths were probably killed by high salinities caused by drought conditions.

Work was begun on a booklet to aid sportsmen in identifying aquatic plants. Also, three public presentations were made to inform the public about aquatic plant management.

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* The Alabama Department of Conservation and Natural Resources, Game and Fish Division, Spanish Fort, Alabama.

BIOLOGICAL CONTROL TECHNOLOGY

Management of Aquatic Plants with Genetically Engineered Microorganisms

Phase I: Candidate Selection

by
Edwin A. Theriot*

INTRODUCTION

Genetic engineering has developed rapidly into a useful technique with many applications. This technology is used to address problems in such diverse areas as production of pharmaceuticals, treatment of oil spills, control of agricultural pests, and many others. The feasibility of using genetic engineering for biocontrol of aquatic plants was addressed by the Aquatic Plant Control Research Program (APCRP) in 1983. A workshop was held at WES where experts in the field of genetic engineering met to outline the research tasks required for applying genetic engineering technology to aquatic plant problems (Pennington 1986).** The conclusion was that the approach was feasible.

A six-phase approach was developed as follows:

I. Candidate Selection
II. Trait selection
III. Engineering
IV. Bioassay
V. Efficacy Studies
VI. Formulation Development

It was estimated that the project would take 10 to 12 twelve years to complete. The first phase was initiated in FY 86. It addresses the selection of candidate microorganisms to be engineered and will take three years to complete.

The initial, and perhaps most crucial, requirement is finding a host specific microorganism. Specificity denotes the ability to grow in nature exclusively on or within a target plant. Since host specificity usually involves several interactions between the microorganism and host plant, several genes are probably involved. If these genes are widely separated on the chromosomes, extensive manipulations that are impractical at this time would be necessary to make use of the genes. Therefore, the candidate must be intimately and exclusively associated with the target plant.

APPROACH

Three separate efforts are included in the first phase of the project: (a) a research effort to identify microbes that are specific to hydrilla and Eurasian watermilfoil, (b) a research effort to determine mechanisms of specificity, and (c) the establishment of an advisory committee.

Host-specific candidates

A method is needed to determine if microorganisms are specific for a target plant regardless of the mechanism of association. The University of Wisconsin is under contract with the WES to develop a protocol to screen for microorganisms specific to submersed aquatic plants. The working hypotheses are that: (a) microbes isolated from plants segregate by growth potential into colonists or non-colonists, (b) colonists segregate functionally into nonparasitic epiphytes and endophytes, or pathogens, (c) colonists are selective or nonselective for a target plant, and (d) selective isolates can be assigned into resident and transient groups (Figure 2).
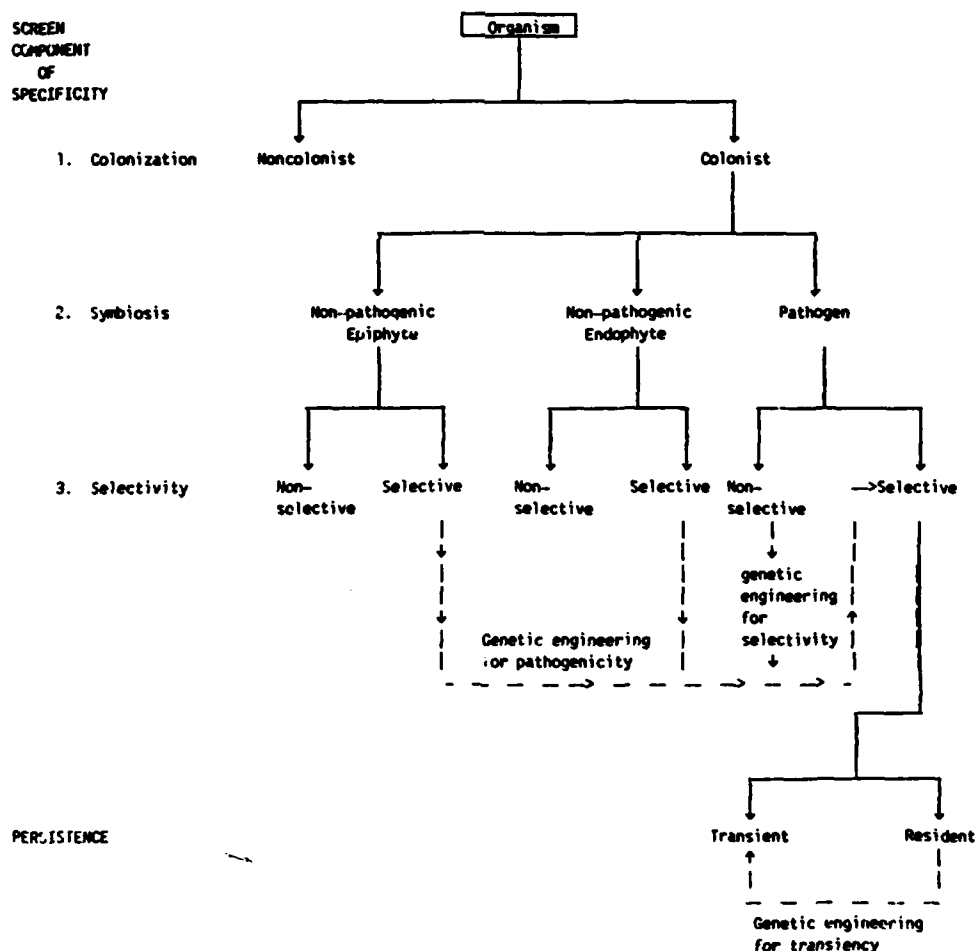

Figure 1. Conceptual and experimental overview of host specificity and persistence of microflora from aquatic plants (after Andrews et al., University of Wisconsin)

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Mechanisms of specificity

Lectins are proteins or glycoproteins that bind to cell surfaces via specific oligosaccharide components. They are common to plants and some microbes. The hypothesis of this study is that lectins provide a site on the plant surface that interacts with a specific, distinctive saccharide molecule on the surface of microbial cells. The WES is conducting studies to isolate lectins from submersed aquatic plants and demonstrate specific attachment to isolates from the plant microflora.

Crude protein components are isolated from Eurasian watermilfoil and Hydrilla. Shoots, roots, and tubers (in the case of hydrilla) are homogenized in a blender, sonicated for 3 min, and centrifuged at 27,000 g's for 30 min. The supernatant solution if dialyzed against a phosphate buffer solution for 24 hr and tested for activity. The protein (agglutinin) is purified by affinity chromatography and concentrated with ultrafiltration techniques. The molecular weight of the protein units is determined by electrophoresis in 10-percent polyacrylamide gel slabs.

The agglutinin in the crude and purified form is tested for activity in fungal spore and hyphae agglutination tests. Agglutination will be determined with phase-contrast and fluorescence microscopy.

Advisory committee

An advisory committee of experts was established to provide guidance. Members include researchers with expertise in plant pathology, bioengineering, molecular biology, ecology and biocontrol technology. The committee will meet once a year to evaluate research results and provide guidance for future efforts. Members will be requested to review proposals and technical reports.

RESULTS

Host-specific candidates

Drs. Andrews, Harris, and associates at the University of Wisconsin have developed a prototype of a flow-through (perfusion) chamber in which isolates of microflora originating from submersed aquatic plants are introduced individually to plants in mineral salts medium (Figure 2). The medium is then exchanged on the order of 0.5 volume per hour and assayed over time for viable propagules. At the termination of the experiment, microbe populations are quantified by indirect (washing plants, dilution-plating, enumeration of propagules) and direct (microscopic) methods to determine the selectivity component of specificity.

A large bank of isolates has been acquired for testing. Stock cultures isolated from Eurasian watermilfoil and maintained at the University of Wisconsin will be screened as well as isolates collected during a national survey and held in culture at WES (Zattau, in preparation).* The screening procedure has just begun; therefore, we have no results on the specificity of isolates.

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* W. C. Zattau. "A Survey of the Continental United States for Pathogens of Eurasian Watermilfoil," Technical Report in preparation, US Army Engineer Waterways Experiment Station, Vicksburg, Miss.
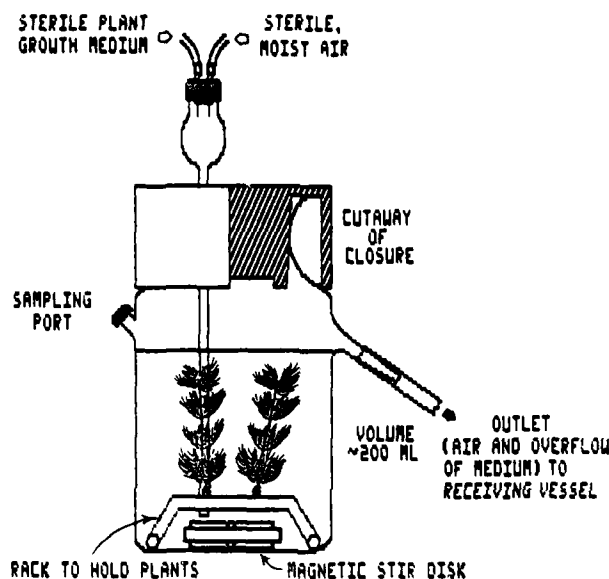
Figure 2. Prototype flow-through culture vessel made from a 300-ml Corning beaker (after Andrews et al., University of Wisconsin)

Mechanisms of specificity

Crude protein complexes have been isolated from shoots, roots, and tubers of hydrilla. Fungal hyphae agglutinin assays have demonstrated the existence of plant protein agglutinins. The molecular make-up of the agglutinins has not yet been identified.

Advisory committee

Four members of the advisory committee have been selected and have agreed to serve. These include: Dr. Tsune Kosuge, University of California - Davis; Dr. George H. Lacy, Virginia Polytechnic Institute; Dr. Steven Lendow, University of California - Berkeley; and Dr. O. C. Yoder, Cornell University. The committee is scheduled to meet for a workshop at WES in April or May of 1987.

DISCUSSION

Host-specific candidates

In host-specificity tests with pathogens, researchers look for symptoms on the target plant. The microbes being screened in this study are components of the target plant microflora. They are normally saprophytic or weakly pathogenic; therefore, they may or may not produce symptoms on the target plant. The perfusion chambers will allow us to identify microbes that grow on, or in, the test plant regardless of symptoms.

Microbes screening positive for growth, on or in, hydrilla or Eurasian watermilfoil will be assayed for selectivity by introduction to perfusion chambers containing two to three nontarget macrophyte species. In complementary control experiments, target plants will be added to some chambers. Selectivity will be indicated by microbial washout followed by negligible counts.

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The chambers will also be useful for: (a) efficacy studies, (b) analysis of the extent and speed of infection, (c) evaluation of the environmental factors affecting infection, and (d) verification that the engineered organism can control the target aquatic plant. These investigations are to be conducted in Phases IV and V of the project.

Mechanisms of specificity

Adhesion of microbes to the plant cell surface is not only a prerequisite to successful colonization, but also determines the type of symbiotic relationship that will occur between the two. In most cases selective adhesion of the microbe to the host cell wall is an early determinant of host specificity.

The identification of lectins or other agglutinins on the surface of hydrilla and milfoil that provide for specific attachment to microbes would be a major accomplishment. It may explain the mechanisms of attachment for those microbes that prove to be host specific. Host specificity could be engineered into a candidate if a lectin determines specificity, since it is likely that a single gene is needed for its production. Microbes could be screened for surface molecules that are specific for the isolated lectin, thereby providing another means of screening isolates.

Advisory committee

The advisory committee will provide valuable guidance to assure that all technical, ecological and regulatory considerations are addressed. There is no doubt that the technical goals of this project can be achieved for the field of genetic engineering is developing rapidly, providing solutions to previously unsolvable problems. The major concern, however, is regulatory requirements for release of engineered microbes into the environment.

Dr. Lendow, U. C. Berkeley, has gained valuable experience in this area. He is presently attempting to gain approval for release of an engineered microorganism that prevents frost damage to crops. The concerns being raised are of an ecological nature. For example, what will be the fate of the engineered microbe in the environment? Will it upset the delicate ecological balance? These problems must be addressed before releases of engineered organisms into the environment will be approved.

Microbial Control of *Hydrilla verticillata*

by
Edwin A. Theriot*

INTRODUCTION

This study is based on the theory that microorganisms, which exist in the natural microflora of hydrilla, have a latent ability to attack the plant when conditions favor the microbe and/or stress the plant. It was modeled after research conducted on Eurasian watermilfoil by Dr. Haim B. Gunner, University of Massachusetts at Amherst (Gunner 1983).**

Previous results have identified 20 fungal isolates that produced enzymes lytic to hydrilla tissues, six of which caused damage to hydrilla in test tube assays (Theriot and Pennington 1985).† Two of the isolates have been eliminated from further consideration; 20 remain. Isolate 224, which is the only isolate to produce pectinase enzyme, failed to demonstrate damage to hydrilla in test tube assays. However, since pectinase production is normally a predisposing factor for infection by many fungal plant pathogens, 224 was included with the other six as primary candidates (Table 1). These seven isolates will be evaluated first in aquaria assays and field studies. Aquaria assays and plant culture techniques have been refined to evaluate candidates on plants growing in lake sediment (Pennington 1985).‡

Table 1
Candidate Microorganisms Evaluated in
Aquaria Assays

Isolate Number	Name
56	*Aspergillus awomori* Nakazawa
156	*Humicolo* sp. with *Tricoderma* sp.
161	*Humicolo* sp. with *Tricoderma* sp.
170	*
224	*
236	*Fusarium moniliforme* Sheldon
249	*Aspergillus awomori* Nakazawa

* Unidentified.

To be effective as a biocontrol agent, a candidate must express latent virulence factors, thrive in conditions favored by the plant, and compete favorably with other components of the microflora. The enzyme production of these candidates was enhanced in hopes that they would become virulent.*

The purpose of this article is to report the results of studies conducted to evaluate growth of candidate isolates under conditions favorable to hydrilla, as well as, efficacy studies of isolates alone and in paired combinations on hydrilla.

APPROACH

Isolate growth studies

Growth experiments were conducted to identify isolates that grow best under conditions common to hydrilla. The candidate isolates were grown at temperatures of 16°, 25°, and 30° C on potato dextrose agar (PDA) plates adjusted to pH 7. Isolate inoculum, taken from stock culture, was placed on the center of each plate with a sterile loop. Growth diameter measurements were taken 5 and 10 days after inoculation. Isolates were also grown at 25° C on PDA adjusted to pH levels of 5, 7, and 9 to determine optimum pH for growth.

Aquaria assays

Aquaria assays of the seven primary candidates were conducted as recommended,* using first-generation field collected hydrilla plants and whole liquid inoculum of the isolates. Eight aquaria were set up, one for each treatment and an untreated control (Figure 1). Nine 8-oz plastic cups containing lake sediment

Figure 1. Control aquarium 2 weeks after study initiated

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* Pennington, op. cit.

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planted with three sprigs of hydrilla were placed in each of the eight aquatic (n = 9). Liquid inoculum was grown in culture roux bottles containing 200 ml of V8 juice broth for 3 weeks at ambient temperature in order to obtain a mixture of both hyphae and spores. The inoculum was blended with a Waring blender, and 600 ml of inoculum was applied to each treated aquarium. The assays were run for 8 weeks.

Isolate combination studies

Isolates were grown in pairs on PDA plates to determine compatibility. Each isolate inoculum was centered on one side of the plate, two isolates per plate. A zone of inhibition (no growth) between isolates on the same plate signified incompatibility.

Compatible combinations of isolates were evaluated in test tube assays using healthy sprigs of hydrilla. One millilitre of each of the two isolates was inoculated in each test tube. The inoculum was prepared in the same manner as was the aquaria assay. Each treatment was applied to 10 test tubes (n = 10). A damage index* was given each test tube once a week for 6 weeks.

RESULTS AND DISCUSSIONS

Isolate growth studies

Results of the growth studies demonstrated that five of the 20 isolates tested grew best at 25° C (Table 2). The remainder preferred temperatures between 25°

Table 2

Growth Studies of Lytic Enzyme-Producing Isolates

Isolate Number	Preferred Temperature (° C)	Preferred pH
049	25	†
056*	30	9
057	25-30	†
059	25	7-9
101	25-30	9
111	30	†
116	25-30	†
156*	25-30	7
157	30	9
161*	25-30	7
162	25-30	5
170*	25-30	7
224**	25	7-9
236	25	†
237	25-30	†
240	30	†
242	25	7-9
244	25-30	†
249*	25-30	†
250	30	9

* Isolates that damaged hydrilla in test tube assays.

** Only isolate to produce pectinase enzyme.

† No significant difference in growth at any pH.

* Ibid.

and 30° C. Mean water temperatures, 6 to 12 in. from the surface, in hydrilla mats of the southeastern United States often reach 25° C in the late summer. Candidates that grow well at 25° C or lower indicate an ability to coexist with hydrilla and compete favorably with other members of the plant microflora.

Hydrilla prefers a water pH of neutral to slightly basic. All isolates grew moderately well at all three pH levels tested. One of the 20 isolates grew best at pH 5, and 10 preferred pH 7 or higher (Table 2). There was no significant difference in growth for the remaining nine isolates tested. It was to be expected that the majority of the isolates grew best at the higher pH levels, since they were all collected from healthy field hydrilla. Seven of the isolates that grew well at the higher pH levels preferred the lower temperatures (Table 2). Three of the seven were isolates that damaged hydrilla in test tube assays; 156, 161, and 170. Isolate 224 also preferred a pH range of 7 to 9 and grew best at 25° C.

Aquaria assay

Two weeks after the aquarium test was initiated isolate 224 had completely destroyed the hydrilla (Figure 2). Plant tissues disintegrated and settled to the bottom of the aquarium. Plant decomposition was accompanied by an algal bloom. The rapid decline and decomposition of tissues would seem to indicate toxin and/ or enzyme affects.

Isolates 224, 56, and 249 achieved maximum shoot length significantly greater than the control, but none exhibited significant increases in above-ground biomass. It appears that 244, 56,and 249 elicited a plant growth hormone reaction, most likely gibberellin production. Gibberellin causes cell elongation in plants, which would explain the increased length without increased biomass. No disease symptoms were observed in any of the aquaria other than the one treated with 224.

Figure 2. Aquarium treated with isolate 224, 2 weeks after treatment

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Isolate combination studies

All 20 isolates tested proved to be compatible with each other. Test tube assays were conducted on all combinations to determine the efficacy on hydrilla sprigs. Three weeks after treatments were applied to hydrilla test tube assays, two combinations exhibited significantly greater damage than the control: 249/170 and 170/59. Mean disease index values for the two combinations were also significantly greater than the individual isolates. The results indicate a synergistic effect.

CONCLUSIONS AND FUTURE RESEARCH

Conclusions

Conclusions of this study are:

- Seven of the isolates demonstrated an ability to grow well at temperatures and pH levels suitable for hydrilla growth.
- Fungal isolate 224 is capable of attacking and destroying healthy hydrilla growing in lake sediment.
- All paired combinations of the 20 lytic enzyme-producing isolates are compatible.
- Two combinations of isolates significantly damaged hydrilla in test tube assays: 249/170 and 170/59.

Future research

Aquaria assays will be conducted to verify the efficacy of 224 and evaluate the paired-isolate combinations. Host-specificity studies will be conducted in aquaria using native submersed aquatic species with 224 to determine its host range. Small-scale field studies will be initiated to evaluate 224. A field site in southeast Texas will be selected because the isolate was collected from Lake Conroe, Texas. Approval for testing the isolate in the area of origin is expected to be given more readily.

Feasibility Study for the Biological Management of Submersed Aquatic Plants by the Manipulation of Phytophagous Invertebrates

by
Ann O. Jones* and Ted D. Center*

OBJECTIVE

A principal objective is to evaluate the potential for manipulation of populations of native and introduced arthropod species through various means directed at increasing their impact as weed control agents. Emphasis will be placed on the potential use of behavior-modifying compounds such as attractants, feeding stimulants, and other secondary compounds which may be present in submersed aquatic plants. The principal objective will be to provide recommendations for or against a future course of research. A secondary objective will be to provide WES personnel with a cross-indexed, accessible data base of all literature records relevant to the above-mentioned recommendations. This will include copies of key articles which most significantly influenced conclusions and recommendations.

INTRODUCTION

Several recent studies at numerous sites have documented population declines of submersed aquatic weeds as the result of infestations of insect herbivores. For example, in Africa at Lake Tanganyika midge larvae attack the apical buds of hydrilla. The larvae destroy many meristems and thereby present upward growth of the plant (Markham 1986). The Canadians have documented cases in which another midge species has a similar effect on *Myriophyllum spicatum* and is able to stop the shoots from growing to the water surface (Kangasniemi 1983). These same investigators also found that weevil larvae had a detrimental effect on *M. spicatum* in Osoyoos Lake, British Columbia. In fact, damage was so extensive that most of the plant canopy had broken away and the remaining plants were badly decayed where larvae had grazed. Painter** documented the decline of *M. spicatum* at Buckhorn Lake in Ontario caused by an infestation of the accidentally introduced moth *Acentria nivea*. Vestjens (1979) described an instance in which a flea beetle *Haltica ignea* destroyed *M. verrucosum* when it was exposed during periods of low water in Australia. Baloch, Sana-Ullah, and Ghani (1980) conducted aquarium experiments which adequately proved the potential for *Hydrellia pakistanae* to control hydrilla. Berg (1950) and Soska (1975a, b) showed that several species of phytophagous insects annually destroy submersed pondweeds

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* US Department of Agriculture, Aquatic Plant Management Laboratory, Fort Lauderdale, Florida.

** Personal Communication, 1986, Scott Painter, Environment Canada, Burlington, Ontario.

(*Potamogeton* spp.) in North America and Europe. Balciunas (unpub. report) described the mowing effect of *Bagous australasiae* on hydrilla at a reservoir in Queensland, Australia. In this case, the weevils burrowed in the stems and caused the loss of the upper portions of the plants down to about a meter below the surface. Poi de Neiff (1979) described the attack of the bagoine weevil *Ilodites lembulus* on stems and leaves of *Egeria naias* in Argentina which sometimes totally devastated the plant populations. Numerous reports have cited the extensive damage to hydrilla caused by various species of nymphuline moths (Lekic 1970; Habeck 1974, 1983; Balciunas and Center 1981; Balciunas and Habeck 1981). Finally, Buckingham and Bennett (1980) noted that the weevil *Bagous affinis* has been reported to destroy up to 100 percent of hydrilla subterranean turions ("tubers") in India and Pakistan. Thus, insects can, and often do, reduce populations of aquatic weeds.

While most of these examples are the result of the accidental introduction of exotic insect species (fortuitous biological control), several represent the natural effects of native insects within the native range of the plant, and a few are examples of control of exotic plants by native insects. In each case, decimation of the weed population has been associated with an accumulation of insects. These insect aggregations may have resulted either from attraction of large numbers to the site or from normal buildup of resident populations within the site. If the former case was true, then perhaps these herbivorous species could be artificially attracted to sites infested with aquatic weeds specifically for the purpose of controlling those weeds. The feasibility of controlling *Hydrilla verticillata* Royle and *Myriophyllum spicatum* L., by the manipulation of phytophagous invertebrates is therefore under consideration. It must be remembered, however, that this presupposes the availability of host-specific phytophagous invertebrates for manipulation. For the most part, these must first be acquired from foreign regions where either weed species is native. The approach described herein, then, must be considered supplemental to a traditional, foreign-based biological control project, and not a replacement for it.

LITERATURE SURVEY

As a first step in this project, we conducted a thorough literature survey. Various computerized literature data bases were searched using the Dialog system and the Sci-Mate data base management software package. Additional references were acquired from bibliographies contained within books, reports, journal articles, etc. Maximum attention was given to literature which dealt with plant-derived insect attractants. Most information available on insect attractants dealt with pheromones (insect sex attractants). Since these function in insect-to-insect interactions (usually to attract males to females), we felt that these would have no applicability in weed management systems. Literature on pheromones was therefore excluded in this review. Various other aspects of the ecological relationships between insects and plants which did not deal with insect attraction were considered important and references to them were retained in the data base.

We have now successfully compiled a literature data base of over 2,000 references

which deal with pertinent aspects of this study. Copies have been acquired of over 90 percent of the references and these are now in our files. We are now involved in the time-consuming process of reviewing and summarizing this literature. A preliminary synopsis of our findings is presented within this report.

REPORT SUMMARY

Hydrilla verticillata Royle, a submersed, freshwater angiosperm in the family Hydrocharitaceae, adapts readily to climatic and edaphic factors and to competition from other plants. The leaves, which are two cells thick, with abundant air lacunae, and four to five cells thick around the central vascular tissue, are specialized for photosynthesis (Benedict 1978). The photosynthetic characteristics of hydrilla are associated with the C3 cycle and an inducible C4-type cycle which allow it to vary photorespiration in response to environmental conditions (Bowes and Salvucci 1983). With low CO_2 compensation points net photosynthetic rates are increased, O_2 inhibition is reduced, and minimal photorespiratory CO_2 is released (Salvucci and Bowes 1981); the reverse occurs in the high CO_2 compensation point mode (Holaday, Salvucci, and Bowes 1980). Hydrilla in its natural habitat photosynthesizes at CO_2 levels below saturation and requires low solar radiation (Van, Haller, and Bowes 1976). Hydrilla grows well in a wide range of nutrient levels, water pH, salinity levels (to 33 percent seawater), water depth, and temperatures (Swarbrick, Finlayson, and Cauldwell 1981). Hydrilla leachates include p-Hydroxybenzoic and vanillic acids, which are classified as growth inhibitors (Rao, Rao, and Rao 1980). Some members of the Hydrocharitaceae have the ability to produce HCN, which is toxic to many herrbivores (Seigler 1981). These factors give hydrilla a competitive advantage over other submersed plants.

Myriophyllum spicatum L., a submersed, freshwater angiosperm in the family Halorgaceae, also adapts readily to climatic and edaphic factors and to competition from other plants. Although Salvucci and Bowes (1983) report data that indicate the low photorespiratory system of *Myriophyllum* is mediated by different mechanisms than the C-4-like mechanisms in hydrilla, it exhibits phytosynthetic characteristics similar to hydrilla (Van, Haller, and Bowes 1976). The species differ in the amount of light required for photosynthesis and in the CO_2 compensation points. The lower light compensation point of hydrilla may contribute to its ability to dominate over *Myriophyllum* and other submersed macrophytes (Grace and Wetzel 1978). Like hydrilla it grows well in a wide range of nutrients, water pH, salinity levels, temperatures, and water depths. Because the critical tissue concentrations for N and P (0.75 and 0.07 percent) are much lower than for other submersed macrophytes, *Myriophyllum* can avoid nutrient limitations.

The factors which attract insect herbivores to submersed aquatic plant species are unknown. This is especially problematic since the host-seeking stage is normally the aerial adult insect. The mechanisms used by these aerial insects to detect submersed plants must then be determined before there can be any hope of managing their populations.

In the aquatic community, a variable complex mixture of chemicals exists, which is dependent on the growing season and related to plant population (Meteyko 1982).

Insects that feed on exotic weeds probably evolved in different circumstances of host-plant permanence and predictability than exists within the adventative range of the weed. Therefore, their search strategy may be quite different (i.e., more efficient) than expected. However, location of hydrilla or *Myriophyllum spicatum* by olfactory receptors of host specific herbivores in close proximity should not be a problem since both have competitive advantages over other submersed aquatic plants and rapidly spread after introduction to a body of water (Grace and Wetzel 1978; Swarbrick, Finlayson, and Cauldwell 1981). Hydrilla has a specific aroma that is distinguishable from other plants. In any area where hydrilla is dominant, its aroma permeates the atmosphere. Hydrilla has been observed to release substances that form an oily, irridescent layer on the surface of the water. These leachates may serve as attractants to insects not only by odor but also by photoemissions or photoabsorbance. Matthews and Matthews (1978) report that the most effective region of the spectrum in directing phototaxes is in the 350-nm range, as demonstrated in the use of ultraviolet lamps in insect traps. The adaptive significance of ultraviolet phototaxes may be that it signals "open space" as the result of photoabsorbance of the longer wavelengths in visible light by chlorophylls. However, stimuli by photoemissions from the plant photosynthetic processes or the interaction of light with secondary products secreted from the plant tissues may be perceived by the flicker vision, which is employed in firefly communication. Because herbivores that feed on hydrilla and *Myriophyllum* spp. usually swarm at night and have been observed to be attracted to light traps, visual receptors as part of the host recognition and location should not be ignored.

Both hydrilla and Eurasian watermilfoil normally have relatively low nutrient, nitrogen, and protein content and high phenolic content (Boyd 1969; Easley and Shirley 1976; Rao, Rao, and Rao 1980; Roe and Bruemmer 1980; Planas et al. 1981). The high phenolic content would be expected to deter most herbivores although various phenolic compounds may serve to attract highly specialized, host-specific species. To determine this, a screening process must be carried out in which natural plant products are extracted, isolated, and identified. These must then be tested to determine which substituents attract the insects and stimulate them to feed.

If the factors which cause the attraction of a herbivore to a particular species can be identified, then it may be possible to enhance attraction to infested areas by artificial means, i.e., introduction of photostimuli and/or olfactory stimuli. If the feeding stimuli and nutrient requirements for growth and reproduction of the herbivore can be identified and quantified, then it may be possible to manipulate the plant metabolism to increase the adequacy of the selected food to sustain growth, survival, and reproduction (Scriber 1984). It may also be possible to enhance herbivore biochemical responses to the target plant by adaptation to the chemical qualities inherent in the plant. Adaptation of a herbivore to specific chemicals in a plant has been accomplished by the introduction of the stimulatory chemicals in progressively increasing amounts to its diet (Feeny 1975; Brattsen and Wilkinson 1977; Scribner 1984). To do this, quantitative work on the effects of the chemicals which influence behavior responses, particularly threshhold ranges of acceptance, must be carried out (Miller and Strickler 1984).

Phytophagous invertebrates do feed on hydrilla and on *Myriophyllum* spp. in various parts of the world (Baloch and Sana-Ullah 1973; Balciunas, unpublished report; Spencer and Lekic 1974; Habeck 1983). Prospects for the biological control of hydrilla include leaf mining flies (*Hydrellia* spp)., nymphuline moth larvae, tuber-boring weevils (*Bagous affinis*) (Balaoch and Sana-Ullah 1973; Baloch, Sana-Ullah, and Ghani 1980), meristem-feeding midge larvae (Markham 1986), and stem-boring weevils (*Bagous australasiae*) (Balciunas 1985). *Paraponyx stratiotata* L., *Cricotopus* n. sp., *Litodactylus leucogaster, Acentria nivea*, and unidentified weevils have been considered for biological control of Eurasian watermilfoil (*Myriophyllum spicatum* L.) (Spencer and Lekic 1974; Kangasniemi 1983). Attractants and feeding stimuli within the hosts have not been identified.

The use of herbivores for biological control of aquatic plants is dependent on the interaction of host specific phytophagous organisms with the metabolites inherent in and released by the macrophytes (Matthews and Matthews 1978, Rosenthal and Janzen 1979). Gurevich (1971, as cited in Meteyko 1982) has shown that all aquatic and littoral plants produce and release metabolites, secondary products, as phytoncides. The types and amounts vary with environmental conditions and with the physiological condition of the plants (Barz and Koester 1981). Insects discriminate between host and nonhost plants by differential behavior responses to various secondary substances (Harborne 1972, Feeny 1975, Bell 1981) and to electromagnetic radiation of light (Prokopy 1983). These behavioral responses are reactions to cues containing biological information which has been accumulated by the different receptors of the insect and interpreted by its central nervous system. Behavioral responses of the phytophagous insect include location, recognition, and acceptance of the host plant (Feeny 1975, Dethier 1976). Host-plant suitability for growth of phytophagous insects is dependent upon their nutritional requirements and adaptation to the plant chemicals (Scriber 1984). Although many secondary compounds are present in all plants and are essential to their survival, chemical constituents have been identified that are unique or distinctive for certain taxa. These compounds which are most useful taxonomically are restricted in distribution and have been found to function in host-insect interactions (Fraenkel 1959, Seigler 1981).

Host-plant attraction, recognition, and acceptance may be accomplished by acoustic, chemical, visual, and/or tactile communication (Matthews and Matthews 1978). Dethier (1976) noted that in lepidopterous larvae, host-plant recognition and acceptance are based upon complex mixed olfactory and gustatory information. He further stated that it is the total chemical complex that forms the basis for perception. Host-plant attraction and acceptance involves gustatory and olfactory receptor responses to cues from both primary and secondary plant metabolites and nutrients (Schoonhoven 1968, Dethier 1976, Hsiao 1976, Jones and Coaker 1978). It appears, however, that most monophagous and oligophagous herbivores that feed on aboveground plant tissue show oriented responses toward secondary metabolites (Jones and Coaker 1978). In contrast, polyphagous species may respond to primary as well as secondary plant metabolites. Attraction to submersed aquatic plants would be expected to occur by olfactory receptor responses to cues from natural compounds released by the macrophyte to the

aquatic environment (Schneider 1969, Dethier 1976, Kennedy 1977, Matthews and Matthews 1978) or by visual receptor responses to cues that result from the interaction of light with matter (Prokopy 1983).

Insect visual receptors respond rapidly to a very small signal of light in the range of 300 to 650 nm, with greatest activities at 350 nm (near-ultraviolet) and 500 nm (blue-green) (Matthews and Matthews 1978). Visual signals include photons which are the result of the interaction of electromagnetic radiation from the sun with matter (Prokopy 1983). The photons may be reflected by plants, their metabolites, and/or their environment to provide visual cues of spectral quality, dimensions, or pattern. Visual signals may also be photons emitted from the living tissue of plants as low-level chemiluminescence, a biochemical generation of electronically excited states, which becomes complex in biological systems and can exhibit an intricate spectral distribution (Amesz and Gorkom 1978). Low-level chemiluminescence of biological systems can be grouped into two categories. One group includes those systems in which the generation of excited species occurs as a consequence of interactions between oxygen-containing radicals; the other group includes those systems in which the excited species are generated in a primary fashion, as direct products of certain enzymatic reactions (Cardenas 1984). The amount of photoemission appears to vary with changes in physiological conditions, e.g., chlorophyll a fluorescence in leaves has been observed to increase when photosynthetic electron transport is inhibited by chemicals or by the reduction of abosrbed light (Habash, Percival, and Baker 1985); the chemilum-inescence of the leaves of aquatic plants increases under the influence of low concentrations of copper nitrate (Kochetov and Tarusov 1975); ultraweak luminescence attributed to nonenzymatic oxidation of tissue lipids increases in the presence of CN ion and abscorbic acid (Shoaf and Steele 1974). Various studies on chlorophyll fluorescence have been made (Shoaf and Steele 1974, Habash, Percival, and Baker 1985) and fluorescence spectra have been used to characterize phytoplankton populations by chlorophyll fluorescence techniques (Yentsch and Yentsch 1979).

Olfactory receptors are stimulated by airborne or waterborne chemical cues which can be very different. Insects respond to the olfactory stimuli interpreted to be acceptable as food, prey, or sex signals (Schneider 1969). Gustatory receptors respond to chemical stimuli transported in water. Responses to specific stimuli have been observed for sugar, salt, amino acids, and secondary plant substances (Schoonhoven 1968).

Host-plant recognition and acceptance depend upon the interpretation of complex chemical information by receptor systems which are made up of different receptor cells. Recognition is the result of chemosensory response by the receptor system to stimuli from the total chemical complex. The chemicals that stimulate a receptor cell and elicit a response are a relatively narrow spectrum of compounds to which the cell is specific. All information is transferred to a central nervous system for integration. (Schneider 1969, Dethier 1976). Chemosensory systems include olfactory, gustatory, and photosensory receptors (Matthews and Matthews 1978). The major classes of natural products involved in plant-insect interactions include terpenoids, nitrogen compounds, phenolics, sugars, and acetogenins. The

biosyntheses of these compounds is the result of "ecological biochemistry" which is dependent upon both the species and its biochemical adaptation to interactions with the environment (Harborne 1977). These compounds are generally classified as plant secondary products, whose synthesis and accumulation can be an endogenously controlled, development-dependent differentiation process and/or can be regulated by exogenous factors as light, temperature, or wounding (Brattsen and Wilkinson 1977, Wiermann 1981, Meteyko 1982). Secondary products are usually synthesized from the primary molecules which constitute the central metabolism of the living cell. The most important starting material is acetic acid (as acetyl-coenzyme A) which by a variation in the fatty-acid biosynthesis leads to acetogenins, terpenes, and steroids. Acetogenins include antibiotics, essentials oils, phenolic compounds, quinones, pigments, and longchain hydrocarbon alcohols and ketones (Hendrickson, Cram, and Hammond 1970). Stafford (1981) proposes that secondary products in higher plants are synthesized in the endoplasmic reticulum membrane by means of a multienzyme complex which are aggregated by covalent bonds. The other major sources are amino acids, particularly lysine, tryptophan, and tyrosine, which lead to alkaloids (Hendrickson, Cram, and Hammond 1970).

Host-plant suitability involves nutritional requirements as well as the ability of the insect to adapt to the plant chemical and physical qualities (Scriber 1984). The acceptability of the host plant is dependent on the presence of phagostimulants and nutritive materials, both kinds and relative amounts, which are required for survival and reproduction (Schoonhoven 1968). Essential nutrients that are available in most green plants and stimulate gustatory receptors on contact include sugars, salts, amino acids, and water (Schoonhoven 1968, Scriber 1984). Harborne (1977) has classified the chemical factors of feeding attractants as essential oils, flavonoids, coumarins, terpenoids, glycosides, and alkaloids. These secondary chemicals may be released from living leaves as volatiles and leachates or by sloughing off of dead tissue (Putnam 1983). A number of feeding attractants and stimuli for monophagous insects have been identified: essential oils (sinigrin) in Cruciferae; essential oils (methyl chavicol, carvone, coriandrol) in Umbelliferae; triterpenoid (sapine) in Leguminosae; glycolalkaloids (capsaicin and nicotine) in Solanaceae (Fraenkel 1959); and a mixture of five essential oils, two flavonoids, a terpenoid, and two sugars in Moraceae (Harborne 1977). The stimulants for host specific insects usually are deterrents and/or toxins for other herbivores. Detoxification may be a biochemical enzymatic process which adapts the herbivore to tolerance of and possibly preference for the inhibitory chemical. Brattsen and Wilkinson (1977) report that mixed-function oxidases (MFO) of a polyphagous insect larva, (the southern armyworm, *Spodoptera eridania*) play a major role in protection against chemical stress from secondary plant substances. The MFO enzymes of an animal appear to be induced by secondary plant substances in its food. Krieger, Feeny, and Wilkinson (1971) concluded that the evolution of the mixed function oxidase system may be induced by exposure to secondary plant substances which include alkaloids, terpenoids, steroids, glycosides, and aldehydes.

Insect acceptance depends on its ability to utilize the plant in its environment and determines the effectiveness of survival at the expense of the plant (Fraenkel

1959). Plant resistance to attack by herbivores include secondary metabolites that are inherent in or that are synthesized by the plant as the result of feeding (Edwards and Wratten 1983). Adaptations of the insect to the plant may be enhanced by exposure to the stimuli in increasing amounts which could induce increased enzyme activity and lead to dependence of the insect for the stimulant (Krieger, Feeny, and Wilkinson 1971). Requirements for stimuli specific to plant species have been observed to enhance attraction and feeding (Fraenkel 1959). Most plant-derived attractants ellicit responses by females rather than males. Females, however, may be responsive only at some critical stage in their life-cycle which is usually related to their physiological age, their mated status, and their oviposition cycle (Finch and Skinner 1974, Hawkes 1975, Hawkes and Coaker 1979, Finch 1980).

Identification of chemicals that attrach and/or stimulate feeding is possible only by isolation of the different classes of chemicals from plants growing under varied conditions and evaluation of response by herbivores which have been observed to feed on a species. The extraction, separation, and characterization of secondary plant products have been outlined by Mabry and Ulubelen (1970), Harborne (1973), Robinson (1975), Mabry, Markham, and Thomas (1980). Identification of many different secondary plant products has been made by thin layer chromatographic analyses (Wagner, Bladt, and Zogainski 1984). However, since attraction may result from complex mixtures of compounds (Dethier 1976) bioassays of isolated compounds may produce confusing results. Prior to this stage, it must be determined that an oriented response by the insect to the host-plant does indeed occur (Finch 1980).

Evaluation of isolated compounds as stimuli to insects which feed on specific plants is possible by behavioral and electrophysiological studies. In electrophy siological studies the electroantennogram may be used to measure the summed receptor potentials, which is a temporary change in the electric charge of a membrane in a sensory cell responding to a chemical stimulus. Microelectrode probes placed in various parts of the insect's antennae and brain receive the signals which are amplified and displayed on an oscilloscope (Matthews and Matthews 1978). Action potentials and receptor potentials from antennal sensilla have been recorded to determine differences in specificity between receptors. The response in each cell is expressed by an increase or a decrease of its spontaneous activity and forms a reaction pattern which is typical for a particular odor (Schoonhoven 1968). Electrophysiological studies of the responses by olfactory and gustatory receptors have been made on different species to determine the chemical complex which serve as attractants and/or stimulants for feeding, ovipositing, and digestion (Schoonhoven 1968, Schneider 1969, Dethier 1976, Matthews and Matthews 1978). Measuring responses to visual and olfactory stimuli provides a means to objectively select feasible methods for the management of submersed aquatic plants by the manipulation of phytophagous invertebrates.

Effective and practical biocontrol of *Hydrilla verticillata* and *Myriophyllum spicatum* involves several steps. Finding the host-specific herbivores, determining the factors which attract the herbivore to the plant, isolating plant chemicals and identifying the ones which are not the usual secondary plant and products,

evaluating the response of the herbivore to these chemicals, investigating the possibilities of using chemicals determined to be attractants to enhance herbivore-plant interaction and to adapt other herbivores to the target plants, and evaluating the effects of electromagnetic radiation on the insect and the interaction of the insect with the plant are but a few of the steps involved in a comprehensive study.

SUMMARY OF RECOMMENDATIONS

Faunal inventories for herbivores which appear to be host-specific to the target weed species are essential. Although a number of herbivores do feed on hydrilla and *Myriophyllum spicatum*, the mechanisms which induce host-specificity have not been established. The most promising insects should be evaluated in the laboratory and then in the field to elucidate mechanisms responsible for host-specificity. Bioassays must be conducted to determine that the insects are attracted to whole plants prior to testing of extracts and isolated compounds.

Plant metabolites should be isolated from the plants at different stages of development while growing under different environmental conditions by standard biochemical procedures. The plant material from representative sites should be collected monthly, stored immediately under nitrogen at -4° C, lyophilized, and ground. Nutrients and secondary plant products, extracted by appropriate methods, should be separated, identified, and quantified.

Herbivores which have been determined to be specific to each of the two species in the field should be evaluated for behavioral responses to plant extracts and isolated plant secondary products. The responses should be measured and recorded by electrophysiological methods that have been used in other herbivore studies and by methods that will be developed as required. Nonspecific herbivores and those specific to plants with toxic secondary products should be evaluated in the laboratory under controlled conditions for attraction and adaptability to the two species and their metabolites. Evaluation of artificial diets containing chemical attractants and feeding stimuli should be made on host-specific herbivores and on nonspecific ones. Suitable diets may be used to induce the other herbivores to feed on the target plants.

The effects of different wavelengths of light on the bioluminescence of the target plant should be determined. The methods for detection and measurement will be developed. The response of herbivores to the irradiated plant should be evaluated, both with and without chemical attractants.

The effects of light, particularly in the near ultraviolet range, on the attraction of herbivores should be investigated. The light should be both continuous and intermittent emission in wavelengths from 350 to 700 nm. Different discrete wavelengths should also be used to determine the most effective attractant. The visual response by the herbivore should be measured to determine minimum amount and maximum distance required for detection.

Evaluating the effects of plants treated with different growth regulators on the attraction and feeding behaviors of the host specific herbivores could provide

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information that would allow manipulation of plant metabolism to enhance biocontrol.

The minimum time required to carry out these investigations and to verify the results for one insect on one species of plant would be three years. It is proposed that emphasis be placed on determining visual attractants. The cost and relative ease of installing the light attractants for herbivores make this the most feasible means of manipulating biocontrol agents. Furthermore, there is potential to adapt this method for use in the control of other plants. Detection and isolation of chemical attractants would require the greatest amount of time and would be much more expensive.

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Australian Insects to Control Hydrilla

by
J. K. Balciunas*

OBJECTIVES

The object of this project is to locate, test and evaluate insects in Australia which might prove to be useful as biological control agents of hydrilla (*Hydrilla verticillata*). Our main objectives during 1986 were: (a) to maintain colonies of the hydrilla-feeding weevil *Bagous australassiae* (Figures 1 and 2), (b) to determine the life history and biology of this weevil, (c) to learn the geographical and seasonal distribution of this weevil, and (d) to ascertain the host range of *B. australassiae* both in the laboratory and in the field.

Figure 1. *Bagous australasiae* adult. During 1986, research in Australia was focused on this hydrilla-damaging weevil. Permission to import *B. australasiae* into US quarantine facilities was received in January 1987
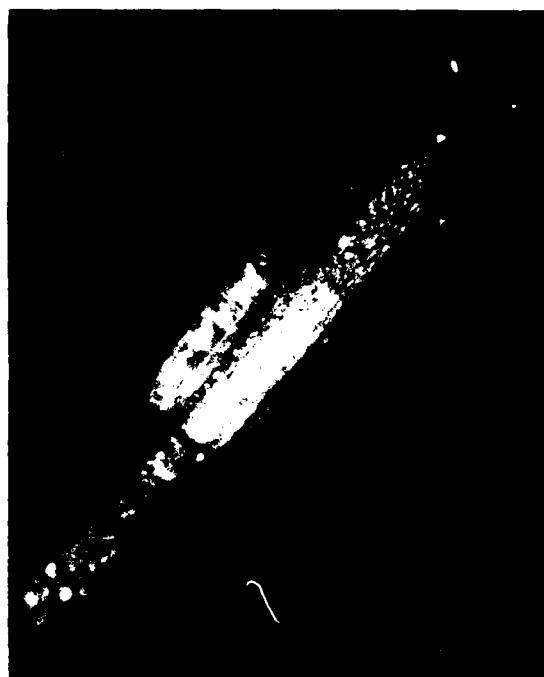

Figure 2. Mature larva of *B. australasiae*. These larvae burrow inside hydrilla stems. The damage from high densities of larvae and external feeding of the aduls causes a fragmentation and disappearance of the top layer of hydrilla at some sites in Australia

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APPROACH

This research program was based at the Commonwealth Scientific and Industrial Research Organizations (CSIRO) Davies Laboratory in Townsville, Queensland (approximate latitude 19° S.) on the northeast coast of Australia. Supplemental research funds along with a record high exchange rate for US monies combined to permit the continued employment of a technician based at CSIRO's Longpocket Laboratory in Brisbane (latitude 28° S.), located 1,000 miles south of Townsville (see map in Figure 3). During 1986, short collecting trips were made to Darwin, Mt. Isa, and along the eastern coast between Sydney and Brisbane. Besides helping to determine the geographic range of hydrilla herbivores, this fieldwork increased the number of potential host plant species examined, and provided additional sources of weevils and host plants.
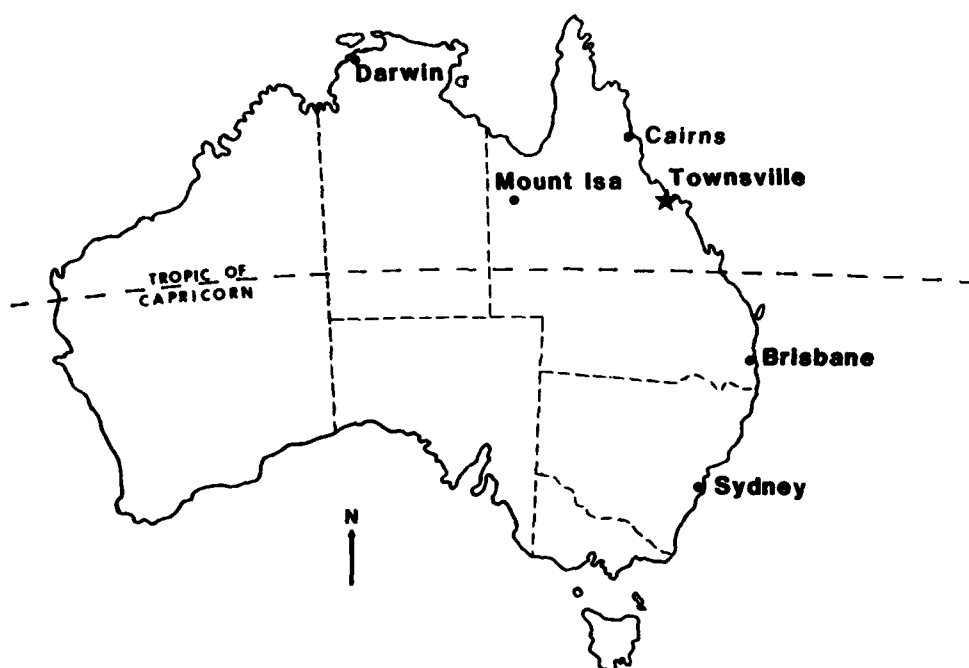
Figure 3. Map of main collection areas in Australia. Research was centered at Townsville, with an additional technician conducting studies in Brisbane

To determine the seasonal variation in the abundance of the weevil and other hydrilla insects, hydrilla from each of six sites in the Townsville-Cairns region and a similar number in the Brisbane vicinity, were sampled on a regular schedule.

Samples of hydrilla and other aquatic plant species were collected in the field and returned to the laboratory for processing. A portion of this plant sample was weighed, then placed in a berlese funnel (Figure 4), to slowly dry, by the heat of a lamp bulb fitted to the top cover of the funnel over a period of 4 to 5 days. The fauna escaping from the drying material was collected daily from the water in a small container placed at the bottom of the funnel. This method proved to

Figure 4. Berlese funnels used to extract herbivores and other fauna from field-collected aquatic plants

be especially effective in collecting stem-boring and leaf-mining insects, such as weevil larvae and *Hydrellia* fly larvae. We attempted to rear most of the immature herbivores to an adult stage, while other fauna was identified (usually only to family or generic level), counted and preserved in 75-percent ethanol.

During 1986, the main emphasis for our laboratory research was host-specificity testing of *B. australasiae*, colonies of which were first established late in 1985 at both Townsville and Brisbane. Most of the weevils from these colonies were then used in our host specificity testing.

Our host-specificity studies of *B. australasiae* consisted of four parts: (a) no-choice feeding tests of adults, (b) no-choice oviposition tests, (c) no-choice larval development tests, and (d) single-choice oviposition tests.

For the first phase of our host specificity studies, we determined the feeding host range of adult *B. australasiae* by confining a pair of weevils in a small plastic cup with a small portion of a single target test plant. After 24 hr we removed the weevils and scored the damage to the test plant on a scale of 0 to 10. Usually 40 to 50 replicates were run simultaneously for each test plant species.

The primary emphasis of our laboratory work during the last quarter was the development of a suitable no-choice oviposition/larval survival host test. The major obstacle was keeping the host plant material in good condition during the 3-week period required to complete larval development. While time-consuming and labor-intensive, the test protocol which we found to be most successful was to expose 10 pairs of *B. australasiae* adults to 20 g of the target host which had been placed on moist paper toweling in a covered plastic box (19 × 19 × 12 × cm). After 3 days the adult weevils were removed, and the number of eggs oviposited in or on the target host were counted. The plant material with the *B. australasiae* eggs

was then returned to the container. The status of the plant material was monitored daily, and fresh material was added as necessary. Dead larvae were counted and removed. After 2 to 3 weeks, the late-third instar, prepupae, and pupae that appeared on the moist toweling were removed and placed in petri dishes containing a moist sterilized soil/vermiculite mixture. As the adults emerged in the petri dish, they were counted and preserved. Since each test took over a month, we restricted the study to plant species that had shown some damage in the feeding tests. We repeated the oviposition/larval survival tests for each plant species until we had four replicates.

The last phase of our studies, the single-choice oviposition tests, was postponed, because good quality hydrilla for use as a control in the experiments was unavailable. We have, however, initiated a small study on the relationship of hydrilla tissue nutrients, especially nitrogen and phosporus, to the feeding and oviposition behavior of the *Bagous* weevils.

RESULTS AND DISCUSSION

In spite of unusual weather patterns, our field collecting continued at an increased place. By the end of 1986 we had made over 340 hydrilla collections and more than 500 collections from 40 other aquatic plant species. Table 1 summarizes these collections.

Most of the aquatic plant species which were available to us in Queensland have now been utilized in our feeding tests, the results of which are presented in Table 2. While hydrilla showed intensive feeding most of the other members of the Hydrocharitaceae also showed moderate to heavy feeding. Of the other aquatic plant species tested, all showed zero or negligible feeding (average feeding scores <1.0), except for *Cabomba, Ceratophyllum, Najas* and *Potamogeton* which had low level feeding (average scores 1 to 4). These weevils starved to death rather than feed on rice (*Oryza sativa*).

All the plant species that elicited a feeding score >1.0 were then tested for their suitability for oviposition and larval development. The bar graph presented as Figure 5 depicts the average number of eggs oviposited on each of the plant species along with the average number of adults produced from these eggs. Once again hydrilla was heavily favored with 75 eggs/test with these eggs eventually developing into 29 adult weevils. Most of the other Hydrocharitaceae produced between 10 to 50 eggs per test. No eggs were oviposited on plant species with low feeding scores.

In order to better illustrate the suitability of the various plant species as laboratory hosts for *B. australasiae*, we have taken the raw counts from the oviposition and development tests and calculated fecundity (average number of eggs per female per day) and survival (number of adults/number of eggs). These are plotted in Figure 6. The upper right-hand corner should contain the obvious host plants, those eliciting high fecundity and allowing a high percentage of the larvae to develop into adults. Only hydrilla (Hv) meets these requirements, and therefore only it occupies this portion of the graph. The upper left-hand portion of the graph is occupied by plants on which fewer eggs were laid, but with high

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Table 1
List of Field-Collected Aquatic Plants Searched for
Bagous australasiae (through 10 December 1986)

Family	Species	QLD	SQL	NTR	NSW	Total
PTERIDOPHYTA						
Azollaceae	*Azolla pinnata?*	9				9
	Salvinia mollesta	2	1			3
Marsilaceae	*Marsilea drummondii?*	30	1	1		32
ALGAE						
Characeae	*Chara* sp.	7				7
	Nitella sp.	3				3
ANGIOSPERMA						
Monocotyledons						
Araceae	*Pistia stratiotes*	5		1		6
Cyperaceae	*Eleocharis* sp.	3				3
	Cyperus sp.	1				1
Graminae	*Leersia* sp.	1				1
Hydrocharitaceae	*Blyxa aubertii*	4				4
	Blyxa octandra	17				17
	Elodea canadensis				1	1
	Egeria densa		30		4	34
	Hydrilla verticillata	213	112	11		341
	Ottelia alismoides	6				6
	Ottelia ovalifolia	4	7		1	12
	Vallisneria gigantea		4	3?	1	8?
	Vallisneria gracilis	38	36			74
Lemnaceace	*Lemna* sp.	2				2
Najadaceae	*Najas tenuifolia*	46	4			50
Pontederiaceae	*Eichhornia crassipes*	6	1			7
	Monochoria cyanea	6	3			9
Potamogetonaceae	*Potamogeton crispus*	1	8			9
	Potamogeton javanicus	8				8
	Potamogeton pectinatus?		1			1
	Potamogeton perfoliatus		5			5
	Potamogeton tricarinatus	16	1			17
Dicotyledons						
Cabombaceae	*Cambomba caroliniana*	5	2			7
Ceratophyllaceae	*Ceratophyllum demersum*	63	3	2		68
Convulvulaceae	*Ipomea aquatica*	9				9
Haloragaceae	*Myriophyllum trachycarpum*	6				6
	Myriophyllum verrucosum	7	2	1		10
Lentibulariaceae	*Utricularia australis*	11				11
Menyanthaceae	*Nymphoides indica*	32	4	2		37
	Nymphoides crenata	1				1
	Nymphoides mexicana		2			2
	Villarsia reniformis	1				1
Nelumbonaceae	*Nelumbo nucifera?*			1		1
Nymphaceae	*Nymphaea gigantea*	10	1	2		13
Onagraceae	*Ludwigia hyssopifolia*	2				2
	Ludwigia plepoides	15	2			17
Philydraceae	*Lanuginosum*	1				1
Polygonacea	*Polygonum decipiens?*	3				3
Total		594	230	23	12	859

* QLD = tropical Queensland, north of Rockhampton; SQL = southern Queensland; NTR = Northern Territory; and NSW = New South Wales.

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Host Family	Genus	Portion Used	Pop. Site*	No. of Tests	Ave. Score**	Std. Dev.	Range
PTERIDOPHYTA							
Azollaceae	*Azolla*	Whole plant	SQL	16	0.00	0.00	0 to 0
		Whole plant	KLB	30	0.57	0.90	0 to 3
Marsileaceae	*Marsilea*	Leaves and top stem	SQL	20	0.00	0.00	0 to 0
		Leaves and top stem	KLB	38	0.00	0.00	0 to 0
Parkeriaceae	*Ceratopteris*	Leaves	SQL	9	0.00	0.00	0 to 0
		Leaves	KBL	39	0.00	0.00	0 to 0
Salviniaceae	*Salvinia*	Whole 'leaves'	SQL	26	0.00	0.00	0 to 0
		Whole 'leaves'	KLB	39	0.00	0.00	0 to 0
ALGAE							
Characeae	*Nitella*	Whole plants	SQL	34	0.15	0.36	0 to 1
	Chara	Tips	KLB	31	0.10	0.30	0 to 1
		Tips	RRD	24	0.16	0.38	0 to 1
ANGIOSPERMA							
Dicotyledons							
Ceratophyllaceae	*Ceratophyllum*	Leaves and stems	SQL	44	3.89	1.67	1 to 8
		Leaves and stems	KLB	30	1.80	1.45	0 to 6
Cabombaceae	*Cabomba*	Leaves	RRD	28	0.75	1.82	0 to 7
		Leaves	KLB	33	1.82	0.73	0 to 4
Compositae	*Cotula*	Leaves	KLB	65	0.00	0.00	0 to 0
Convolvulaceae	*Ipomea*	Leaves	SQL	14	0.00	0.00	0 to 0
		Leaves	KLB	39	0.00	0.00	0 to 0
Haloragacaea	*M. verrucosum*	Leaves and stems	SQL	42	0.36	0.62	0 to 2
	M. trachycarpum	leaves and stems	SQL	23	0.40	0.70	0 to 2
Menyanthaceae	*Nymphoides*	Leaves	SQL	41	0.00	0.00	0 to 0
		Leaves	KLB	30	0.06	0.25	0 to 1
		Leaves	RRD	27	0.00	0.00	0 to 0
Nelumbonaceae	*Nelumbo*	Leaves	SQL	19	0.00	0.00	0 to 0
		Leaves	KLB	36	0.00	0.00	0 to 0
Nymphaeaceae	*Nymphaea*	Leaves	SQL	22	0.00	0.00	0 to 0
		Leaves	KLB	24	0.00	0.00	0 to 0
		Leaves	RRD	18	0.00	0.00	0 to 0
Onagraceae	*Ludwigia*	Leaves	SQL	43	0.00	0.00	0 to 0
		Leaves	KLB	37	0.00	0.00	0 to 0
Polygonaceae	*Polygonum*	Leaves	SQL	11	0.00	0.00	0 to 0
		Leaves	KLB	30	0.00	0.00	0 to 0
Monocotyledons							
Cyperaceae	*Eleocharis*	Leaves	SQL	20	0.00	0.00	0 to 0
		Leaves	KLB	38	0.00	0.00	0 to 0
	Scirpus	Leaves	KLB	50	0.00	0.00	0 to 0
Graminae	*Rice*	Young leaves	KLB	33	0.00	0.00	0 to 0
	Rice	Roots	KLB	34	0.00	0.00	0 to 0
Hydrocharitaceae	*Blyxa*	Leaves	SQL	11	4.91	1.45	2 to 7
		Leaves	KLB	34	5.29	2.01	2 to 9
	Egeria	Leaves and stems	KLB	35	1.89	1.94	0 to 7
		Leaves and stems	RRD	21	1.38	1.60	0 to 7
		Leaves and stems	SQL	34	2.60	1.92	0 to 7
		Leaves and stems	SQL	24	3.50	1.70	0 to 7
	Elodea	Leaves and stems	KLB	40	1.43	0.90	0 to 4
		Leaves and stems	RRD	23	1.69	1.18	0 to 5
		Leaves and stems	SQL	39	1.79	1.61	0 to 7
		Leaves and stems	SQL	16	4.10	2.10	0 to 8

* Weevil populations are coded as follows:
   KLB = Keelbottom Creek, North Queensland
   RRD = Ross River Dam, North Queensland
   SQL = South Queensland.

** Feeding score was based on a scale of 0 (no feeding) to 10 (maximum feeding).

† Poor quality material.

‡ Tips of leaves and stems.

Table 2 (Concluded)

| Host Family	Genus	Portion Used	Pop. Site*	No. of Tests	Ave. Score**	Std. Dev.	Range
	Hydrilla	Tips (L&S)‡	SQL	37	6.81	1.99	2 to 10
		Tips (L&S)‡	KLB	38	2.50	1.74	0 to 7
		Tips (L&S)‡	RRD	40	2.03	1.54	0 to 6
	Hydrilla†	Tips (L&S)‡	SQL	30	3.70	2.14	0 to 9
		Tips (L&S)‡	KLB	32	4.60	1.98	0 to 9
		Tips (L&S)‡	RRD	20	2.35	1.67	0 to 6
	O. alismoides	Leaves	KLB	21	2.19	1.72	0 to 6
		Leaves	RRD	18	2.89	2.65	0 to 8
		Leaves	SQL	17	3.10	2.12	0 to 7
	Vallisneria	Leaves	SQL	36	4.08	1.95	0 to 9
		Leaves	KLB	39	1.74	1.11	0 to 5
		Leaves	RRD	26	1.90	1.35	0 to 5
Lentibulariaceae	*Utricularia*	Tips	KLB	18	0.11	0.32	0 to 1
		Tips	RRD	16	0.13	0.34	0 to 1
		Tips	SQL	13	0.62	1.32	0 to 4
Najadeceae	*Najas*	Leaves and stems	SQL	27	3.26	1.41	1 to 6
		Tips (L&S)‡	KLB	40	0.93	0.73	0 to 3
Philydraceae	*Philydrum*	Leaves	SQL	4	0.00	0.00	0 to 0
Pontederiaceae	*Eichhornia*	Leaves	SQL	24	0.00	0.00	0 to 0
		Leaves	KLB	40	0.00	0.00	0 to 0
	Monochoria	Leaves	KLB	30	0.00	0.00	0 to 0
Potamogetonaceae	*Potamogeton*	Leaves	SQL	34	0.24	0.61	0 to 3
		Leaves	KLB	22	1.19	0.56	0 to 3
Typhaceae	*Typha*	Leaves	SQL	25	0.00	0.00	0 to 0
		Leaves	KLB	39	0.00	0.00	0 to 0
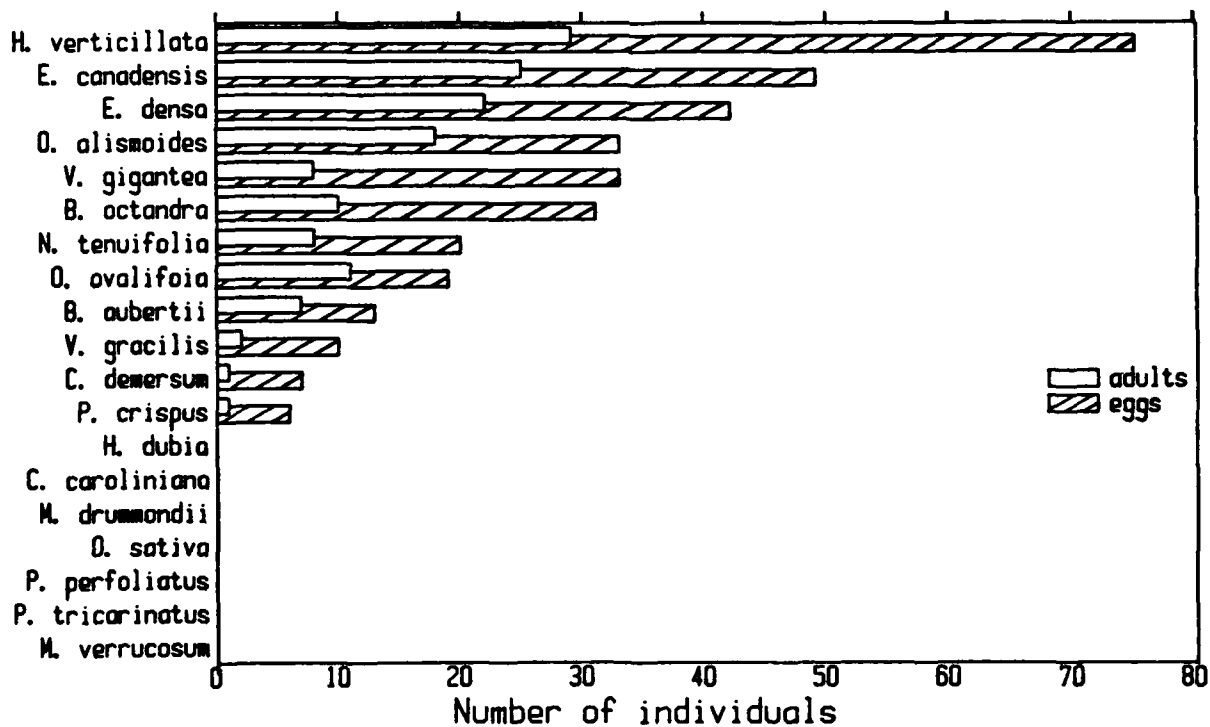
Figure 5. Bar graph depicting average number of eggs and adults of *Bagous australasiae* produced on each test plant species. Abbreviations for each test plant species (see Table 1 for complete names) are on the left
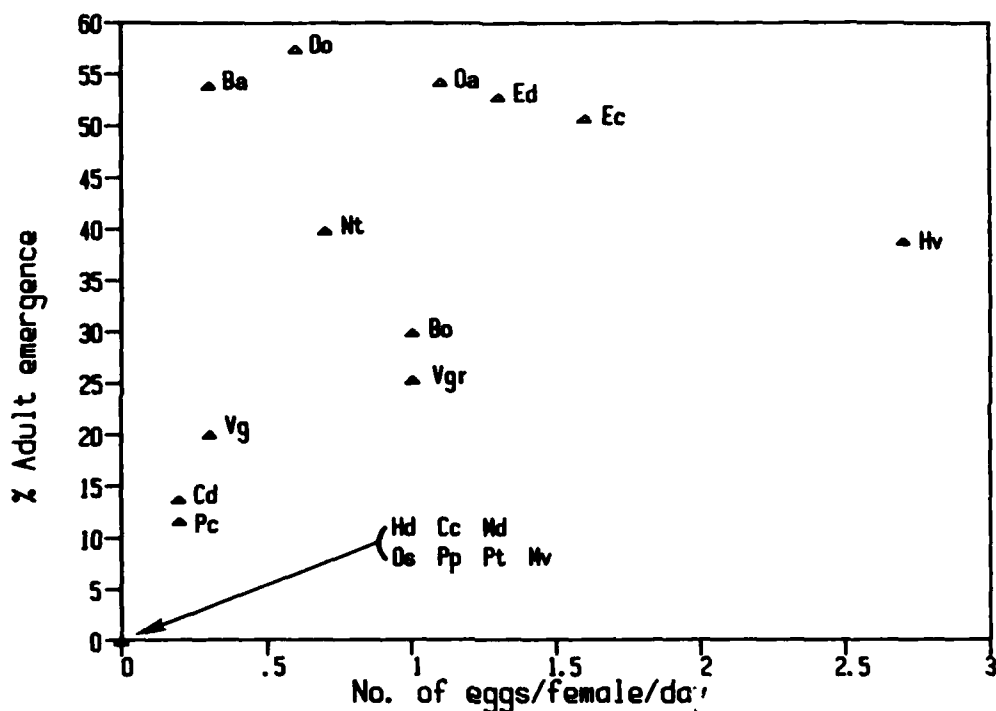
Figure 6. Graph illustrating the suitability of each tested aquatic plant species as a host for oviposition and development of *Bagous australasiae*. Plant species names are coded by the first letter from the genus and species (see Table 1 for list of plant species)

survival of the developing larvae. The plant species *Elodea canadensis* (Ec), *Egeria densa* (Ed), *Ottelia alismoides* (Oa), *Ottelia ovalifolia* (Oo), and *Blyxa aubertii* (Ba) could be considered, on the basis of our laboratory tests, as potential alternate hosts. However, we have made almost 60 field collections of these five plant species, and have not yet recovered *B. australasiae* from any of them. The central portion of this graph, moderate fecundity and survivorship, contains three plant species, *Najas tenuifolia* (Nt), *Blyxa octandra* (Bo), *and Vallisneria gracilis* (Vgr). These plants might serve as possible hosts. However, no *B. australasiae* have been recovered from the 50 collections of *N. tenuifolia* and 17 collections of *B. octandra*. A handful of the 74 collections of *V. gracilis* have produced *B. australasiae* weevils. However, this is an unusual *Vallisneria* species, vegetatively resembling (and frequently confused with) *Blyxa*. *Vallisneria gigantea* (Vg), which more closely resembles our American *Vallisneria americana*, is a poor host, providing low survival for the few weevil eggs that are oviposited on it. No weevils have been found in field collections of this species (Vg) or on *Ceratophyllum demersum* (Cd), or *Potamogeton crispus* (Pc). No oviposition was observed in our laboratory tests of *Hydrocharis dubia* (Hd), *Cabomba caroliniana* (Cc), *Marsilea drummondii* (Md), rice-*Oryza sativa* (Os), *Potamogeton perfoliatus* (Pp), *Potamogeton triicarinatus* (Pt), or *Myriophyllum verrucosum* (Mv).

An interesting point is that no plant species are found in the lower right-hand portion of Figure 6. This helps illustrate the fastidious oviposition behavior of female *B. australasiae* weevils. They refuse to lay eggs on plant species on which chances of larvae developing into an adult are poor.

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This fastidious oviposition extends to hydrilla. We have noted a great variation in the number of eggs oviposited on hydrilla, ranging from no eggs on poor quality hydrilla to over 140 eggs (in 13 days from 10 pairs of weevils) on excellent-quality hydrilla. Our feeding tests have proven to be excellent predictors of the suitability for oviposition of a particular plant sample. Little or no oviposition occurs if a plant produces a feeding score less than 2.5. We have begun to analyze hydrilla tissues for nitrogen and phosphorus, but it is yet too early to analyze the influence of these plant nutrients on the feeding and oviposition behavior of *B. australasiae*.

We have now collected *B. australasiae* from Darwin in far northern Australia, from Mt. Isa in Australia's central desert, and from many locations in Queensland. All previous records for this species have been from New South Wales, along with a few collections near the Murray River in Victoria and South Australia. Thus, this weevil's range in Australia appears to overlap that of hydrilla. Although it has not been recorded from West Australia where hydrilla does occur, I believe that a serious search there would locate this weevil. While the unusual weather patterns this past year make generalizations about this insect's seasonal distribution risky, it appears that the highest populations occur during Australia's spring and summer (October-February).

SUMMARY

Of more than a dozen herbivores discovered feeding on hydrilla in Australia during 1985, our attention has been focused on three species: the weevils *B. australasiae*, the fly *Hydrellia* n. sp., and the moth *Nymphula* prob. *eromenalis*. During 1986, our research concentrated on *B. australasiae*. Large numbers of this weevil cause a "mowing" effect, with all hydrilla within a metre of the surface being removed. A complete life cycle takes 3 to 4 weeks, with larvae burrowing inside the stems of hydrilla. Hydrilla crowns and tubers are also sometimes attacked. It appears that pupation takes place in stranded shoreline vegetation, in shoreline mud, or within submersed hydrilla. Pupation in submersed plants has been demonstrated in the laboratory, but awaits field confirmation.

In laboratory tests, oviposition and larval development of *B. australasiae* occurred only on hydrilla and a few others plants, mostly other species of Hydrocharitaceae. From over 500 field collections of 40 other plant species, immature *B. australasiae* have been found in only six collections of a narrow-leafed *Vallisneria* sp. near *gracilis*. Only negligible oviposition and larval development occurred on *V. gigantea* in laboratory studies. This is a broad-leafed species which more closely resembles the American *V. americana* than does *V. gracilis*. We have never found *B. australasiae* on *V. gigantea* in the field. Thus, it is likely that US *Vallisneria* species will not be suitable hosts.

Our collection data and museum records indicate that *B. australasiae* is distributed from the northern part of the Northern Territory, to Victoria and South Australia. Thus, the distribution of *B. australasiae* appears to overlap that of hydrilla in eastern Australia. The analgous latitudes in the northern hemisphere would be from the mid-Atlantic states to Costa Rica.

B. australasiae seems highly host specific and effective at reducing stands of hydrilla. In addition, it has a short life cycle and is widely distributed. It is an excellent biological control candidate for use against hydrilla in the United States.

Permission to import this weevil into the Gainesville quarantine facility was requested in September 1986 and granted in January 1987. After only 2 years of research in Australia, we will have our first insect in US quarantine, a rate of progress significantly faster than that for most current biological control of weeds projects.

However, it would be unrealistic to expect hydrilla to be controlled throughout the United States by a single insect species. More likely, just as in hydrilla's native range, a complex of agents will be required to achieve adequate biological control of hydrilla.

OBJECTIVES OF FUTURE RESEARCH

The first objective will be to maintain colonies of *B. australasiae* and to ship them to quarantine facilities in Gainesville as required. The second objective will be to continue to gather additional life-history and host-specificity data on *B. australasiae*, especially from the field. The third objective will be to gather additional field host-specificity data on *Hydrellia* n. sp. The fourth objectives will be colonize *Hydrellia* n. sp. in the laboratory and to study its life history and host specificity. This task cannot begin until the *B. australasiae* colonies have been sent to the United States. Since Gary Buckingham has now received permission to release the Indian tuber-feeding weevil, *Bagous affinis*, into the field, this will free up space in the Gainesville quarantine facilities to allow the prompt importation of *B. australasiae*. Limitations of our facilities and personnel allow for only a single species being colonized and tested. The fifth objective (assuming sufficient funds) will be to begin colonizing and testing the moth *Nymphula eromenalis* (Figure 7).

Figure 7. Adult of *Nymphula* prob. *eromenalis*. Larvae of this moth feed voraciously on hydrilla growing in some streams in northern Australia

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Microbiological Control of Eurasian Watermilfoil

by
H. B. Gunner*

ABSTRACT

Field application of the plant-derived microorganisms *M. terrestris* and the pectinolytic bacterium P-8 resulted in the virtual elimination of *M. spicatum* from a treated plot within 10 weeks. An approximately 16-fold reduction of the stem-leaf biomass and a 10-fold decrease in root biomass were observed in samples taken from the treated quadrant compared with those taken from the control quadrant.

Total numbers of bacteria isolated from *M. spicatum* tissues were 3 to 4 orders of magnitude higher than numbers obtained from the adjacent water profile reflecting the substrate commitment of the microorganisms to the plant. Pectinolytic bacteria occurred in greater numbers on treated than on untreated tissues.

Fungal numbers were also higher on treated than on untreated samples and *M. terrestris* was isolated from treated samples throughout the course of the experiment.

Specificity tests of *M. terrestris* infectivity showed it to be very weakly pathogenic to a number of legumes in the seedling stage but not in the mature stage. Oats in the seedling stage showed a higher level of tolerance than in the mature stage. While one variety of bluegrass was tolerant in the mature stage, two were more resistant to infection in the seedling stage. In general the high inoculum level necessary to generate infection reflects the minimal potential the fungus has for significant pathogenic impact outside the area of designated application.

Phosphorus levels were observed to decline in the treated quadrant. However, in the control, and where mechanical harvesting had occurred, the rooted residues appeared to act as a pump to release sediment phosphorus to a level sufficient to initiate massive algal growth in Stockbridge Bowl.

INTRODUCTION

Background and review of previous work

Management methods for the control of Eurasian watermilfoil based on the manipulation of biological systems have shown great potential advantages over other methods (Schuytema 1977). A variety of organisms have been tested for use as biocontrol agents against *M. spicatum*. A snail, *Pomacea australis*, and the

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manatee *Trichechus manatus*, were reported to be potent control candidates (Blackburn, Sutton, and Taylor 1971), but no further report has been given of their use (Amundsen and Brenkert 1978). Among 25 insects found to be associated with *M. spicatum*, *Paraponyx stratiotata* was the only one with sufficient host specificity to be considered for use as a biological control (Spencer and Lebic 1974). Two species of fish, *Tilapia zillii* and the grass carp or white amur (*Ctenopharygodon idella*), have been used for aquatic weed management (Blackburn, Sutton, and Taylor 1971; Amudsen and Brenkert 1978). The inability of the former to survive low temperatures and the potential for the upset of ecological balances by the aggressive grass carp are significant drawbacks which have restricted the use of herbivorous fish as biological control agents (Blackburn, Sutton, and Taylor 1971; Bates, Burns, and Webb 1986).

Plant pathogenic organisms have also been tested for their ability to infect *M. spicatum* (Hayslip and Zettler 1973). Two fungi, *Fusarium sporotrichoides* and *Acremonium curvulum*, were reported to attack *M. spicatum* (Andrews and Hecht 1981; Andrews, Hecht, and Bashirian 1982; Patlak 1982). Although *F. sporotrichoides* can cause localized symptoms and *A. curvulum* can kill the plant under specific environmental conditions which increase plant susceptibility (Patlak 1982), the limitations on the effectiveness of these organisms suggest that further study of the interactions of the plant, its microflora, and environmental conditions is necessary.

Work conducted in our laboratory has taken an ecosystems approach to the development of microbial control agents in which the emphasis is on organisms naturally present in the plant environment. Microflora native to the *M. spicatum* phyllosphere have been screened for the production of enzymes destructive to selected plant components, cellulose and pectin, as well as for their ability to generally stress the plant either alone or in concert. The concentration that this search among plant-associated flora can identify organisms which, after appropriate growth procedures and inoculation back onto the plant, are capable of causing its decline and death has been confirmed (Gunner 1983). A series of experiments in the laboratory has resulted in the selection of two promising microbial control agents, a fungus (*Mycoleptodiscus terrestris* (Gerdemann) Ostazeski) and a pectinolytic bacterium (Isolate p-8), for further investigation (Gunner 1983, 1984). Studies of plant-microbe interactions have demonstrated that the selected species occupy an econiche determined by the competitive advantage provided by their resistance to inhibitory substances released by the plant (Gunner 1984).

Results reported on a preliminary field study (Gunner, Limpa-amara, and Weilerstein 1985) have now demonstrated the potential of the joint application of these two organisms to effectively control *M. spicatum* in nature.

Purpose and scope

The objectives of the work reported below were:

a. To extend the scale of the field study on the efficacy of the microbial agents in controlling *M. spicatum*.

b. To establish the pathogenic specificity of the effective strain of *M. terrestris* particularly on the crop plants recorded as host species.

c. To identify additional environmental variables relevant to the physiological cycle of *M. spicatum* and its decline.

MATERIALS AND METHODS

Preparation of inocula

Fungal inoculum. Mycoleptodiscus terrestris (Gerdemann) Ostazeski (Ostazeski 1967), a cellulolytic fungus, was propagated in potato dextrose salt broth (Gunner, Limpa-amara, and Weilerstein 1985). Thirty-litre batches of culture were grown in a Pittsburg-Des Moines fermenter (40-*l* capacity) at approximately 28° C for 128 hr. Each batch of the culture obtained ws allowed to settle at 5° C in the dark and the excess supernatant discarded. The separate batches were blended at low speed in a Waring blender for 1 min and then pooled to provide a single concentrated culture prior to inoculation.

Bacterial inoculum. A pectinolytic Gram-negative rod, Isolate P-8, was grown in trypticase soy broth (TSB) in aerated containers as previously described (Gunner, Limpa-amara, and Weilerstein 1985). The pooled batches of culture were mixed with 1-percent xanthan gum (Sigma Chemical Co., St. Louis, Mo.) prior to inoculation.

Cell counts

Microbial populations were enumerated by the serial dilution method. Total bacterial counts were made on trypticase soy agar (TSA), while pectinolytic bateria were counted on pectin agar (PA). Both bacterial counts were made after 48-hr incubation 28° C in darkness. Fungal counts were made on Martin agar (MA) after 96-hr incubation at 28° C.

Visual evaluation of plants

The index of plant decline was assessed by visual evaluation of the experimental sites. The condition of the plants was rated on a scale of 0 to 3, with 0 indicating healthy growing plants and higher numbers representing the progressive decline associated with varying degrees of change in the following characteristics: internode elongation, discoloration, turgidity, change in leaf structure, and new growth. The new growth, indicating plant recovery, was weighted against the rest of the categories by subtraction.

Specificity testing

Testing of pathogenic specificity of *M. terrestris* was performed on terrestial plants previously identified as hosts (Gerdemann 1953, 1954; McVey and Gerdemann 1960; Charudattan and Conway 1976). Ten replicates of seedling and mature stages of red clover (*Trifolium pratense* L.), white clover (*Trifolium repens* L.), three varieties of alfalfa (*Medicago sativa* L.), three varieties of bluegrass (*Pao pratensis* L.), oats (*Avena* sp.), and soybean (*Glycine max* L. merr.) were inoculated with 2.1 × 10⁵ CFU/ml of *M. terrestris* using a brush to assure the presence of

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inoculum on the plant tissue. The numbers of susceptible plants, infected and dying, were determined at 2-week intervals for a period of 8 weeks.

Field experiment

Stockbridge Bowl, the experimental site, is a hard-water lake located in Berkshire County in western Massachusetts. The Bowl is infested with *M. spicatum* in areas less than 6 to 7 m in depth. *M. spicatum* growth was continuously removed by mechanical harvester from late spring to early fall.

Two sites, one treated and one control, 2 to 2.5 m in depth approximately 100 m apart were chosen in the southeastern area of the lake. Each site was structured as an octagonal quadrant covering 15 m^2. The external frame of the quadrant was constructed of 4-in. polyvinyl chloride (PVC) pipe that floated freely on the surface of the water. A 4-mm clear plastic curtain suspended from the frame resting on the surface and reaching the lake bottom allowed for better retention of inoculum. An inner structure of 0.5-in. PVC pipe was inserted in the sediment to stabilize the floating frame and curtain surrounding the test area.

The inocula were prepared as previously described and transported to the field site in 15-l carboys packed in ice.The first inoculation consisted of 125 l of bacterium P-8 in 1-percent xanthan at a concentration of 1.1×10^7 CFU/ml and 150 l of *M. terrestris* at a concentration of 2.0×10^3 CFU/ml using the sprayer shown in Figure 1. The unit delivered inoculum at the rate of 4 gal/min at 200 psi through four spray nozzles held approximately 0.5 m below the surface. The second inoculation, 6 weeks following the first application, consisted of 125 liters of bacterium P-8 in 1-percent xanthan at a concentration of 2×10^7 CFU/ml and 96 l of *M. terrestris* at a concentration of 1.2×10^4 CFU/ml applied with the spray manifold apparatus (Figure 2) previously described (Gunner, Limpa-amara, and Weilerstein 1985) at pressures of ca. 5 psi just under the surface of the water.

Figure 1. Spray unit used for first inoculation (designed by Eddie Knight, USAE District, Jacksonville; Palatka Area Office)

Figure 2. Spray manifold constructed of PVC tubing with six 2-mm aperatures used for second inoculation

Microbial populations on the plant surface and in the water profile were measured weekly for 14 weeks. The plant and water grab samples were collected randomly from each test plot and transported in separate containers packed in ice. Microbial counts were performed on the day following sampling. Visual evaluation of the condition of the plants in the test and control quadrants was also carried out weekly for the same period. At the termination of the experiment, eight samples of plant biomass were collected from each test plot, each sample representing plant material from an area of 0.25 m^2. Samples were pulled from the marked area by a scuba diver. Excess sediment and foreign objects were washed from the plant samples before separation of stem and root sections. Each sample was over-dried at 105° C until the weight remained constant.

Phosphorus analysis

Two water grab samples were collected randomly at weekly intervals from the test plots and transported to the laboratory in containers packed in ice. The dissolved orthophosphate was determined by a single reagent method (US Environmental Protection Agency 1983).

Statistical methods

Statistical analysis of data was performed using the F-test for the determination of overall variance and the t-test to determine if significant differences existed between treatments. Analysis was performed by computer application of BMDP statistical programs. The level of significance was determined within a 95-percent confidence interval ($p \leq 0.05$).

RESULTS

Visual evaluation of plants

Visual evaluationof *M. spicatum* (Figure 3) subsequent to the first inoculation showed a slightly higher degree of deterioration in treated plants than in control plants. The decline elicited by the second inoculation in the treated quadrant, however, was up to 5 orders of magnitude higher than in the control, clearly indicating a very high degree of physiological disruption in the treated plants. Within the degraded tissue at the bottom of the treated plot, a few young tips were present but none were visible from the surface of the water even at the termination of the experiment. The decline observed initially in the control plot was dictated by the natural physiological cycle of the plant; however, no further significant visual changes were observed after the seventh week of the experimental period.

Biomass

The effects of treatment on the biomass of Eurasian milfoil are shown in Table 1. An approximately 16-fold reduction of the stem-leaf biomass and a 10-fold decrease in root biomass were observed in the samples taken from the treated quadrant compared with those from the control. The biomass of stem-leaf tissue in the control quadrant was 26.7 times greater than its root biomass, while only 17.2 times higher in the treated quadrant.
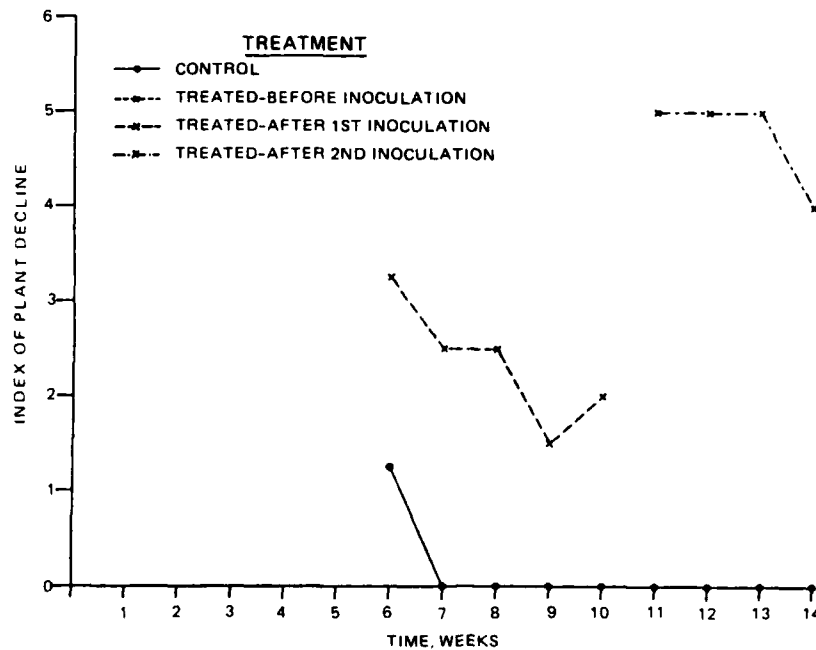

Figure 3. Visual evaluation of *M. spicatum* in control plot and plot treated with *M. terrestris* and bacterial Isolate P-8

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Table 1

Effect of Treatment* on Biomass of Eurasian Watermilfoil in Stockbridge Bowl, Mass.

	Mean Biomass* Dry Weight Plant Material (g/0.25 m²)		
Plant Part	Control	Treated	p-Value
Stem-leaf	133.99	8.23	0.0003†
Root	5.02	0.48	0.0036†
Combined	136.29‡	8.71	0.0020†

* M. terrestris and pectinolytic bacterial Isolate P-8 applied as a biological control agent.

** Average weight of dried plant materials from eight samples/0.25 m²/plot after 10 weeks.

† ≤ 0.01, highly significant.

‡ Discrepancy in total of stem-leaf plus root is due to loss of root tissue in one control sample.

Microbial population dynamics

It will be noted from Figures 4 and 5 that the total numbers of bacteria isolated from M. spicatum tissues remained 3 to 4 orders of magnitude higher than the numbers observed in the adjacent water profile. Total bacterial counts from the water profile reflected virtually a steady state throughout the experimental period (Figure 5). The one perturbation evident occurred following the second inoculation; however, the additional numbers of bacteria declined to baseline levels within 3 days. The bacterial counts from plant tissue gradually increased following the first inoculation. The second inoculation sustained numbers at a somewhat higher level.
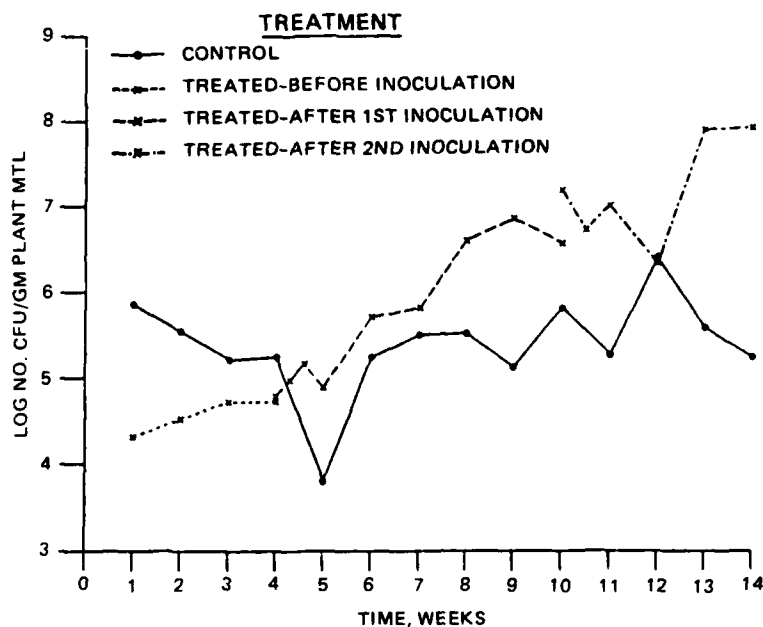

Figure 4. Microbial populations recovered on TSA medium from control and treated M. spicatum tissues in field experiment
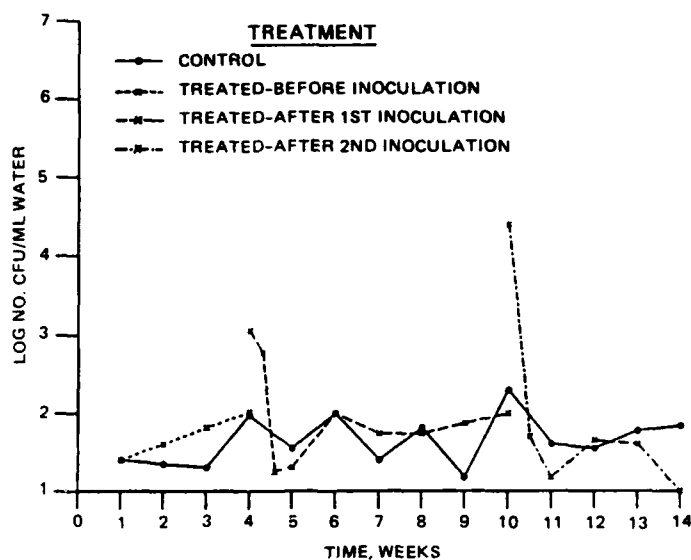
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Figure 5. Microbial populations recovered on TSA medium from water profiles of control and treated plots of *M. spicatum* in field experiment

The numbers of strongly pectinolytic bacteria in the water profile from plant tissue (Figures 6 and 7) showed distribution patterns similar to those of the total bacterial counts in that the numbers on plant tissues were significantly higher. Although the control tissues showed a higher level of strongly pectinolytic bacteria at the outset of the experiment, following the application of the first inoculum, the populations on the treated tissue increased to levels higher than those of the controls, and rose even more sharply subsequent to the second inoculation.

Fungal population numbers (Figures 8 and 9) were low in the water profile, in some instances dropping below the detectable level of the plate count method
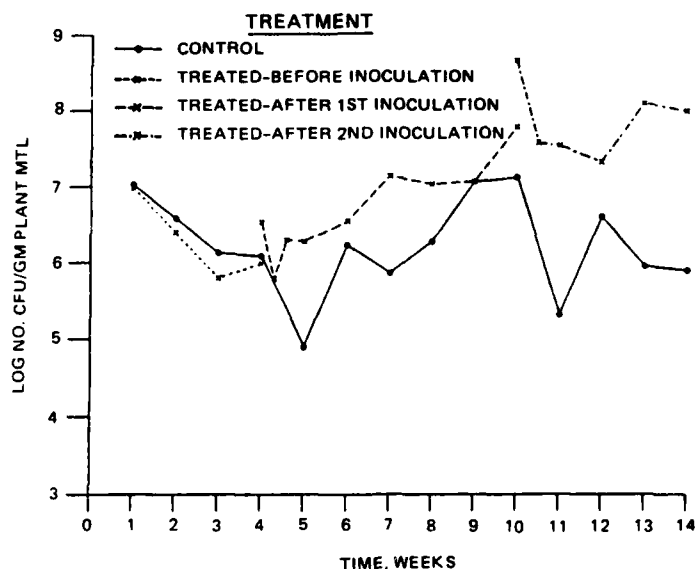

Figure 6. Populations of strongly pectinolytic bacteria recovered on PA medium from control and treated *M. spicatum* tissues in field experiment
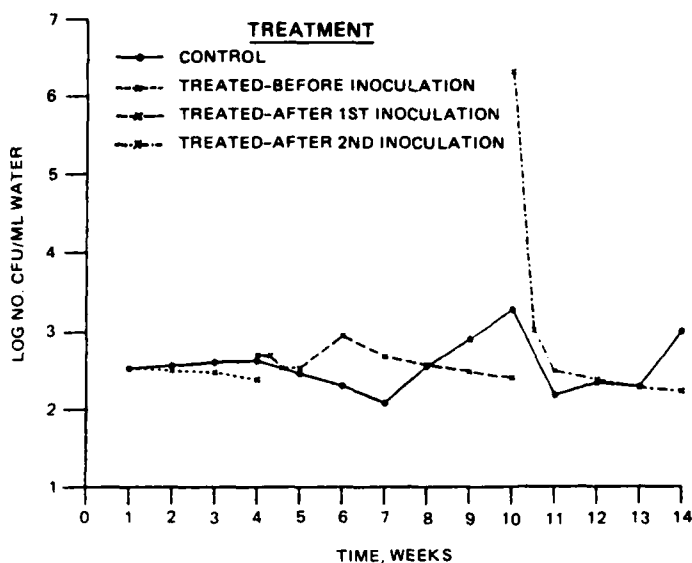
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Figure 7. Populations of strongly pectinolytic bacteria recovered on PA medium from water profiles of control and treated plots of *M. spicatum* in field experiment

Figure 8. Fungal populations recovered on MA medium from control and treated *M. spicatum* tissues in field experiment
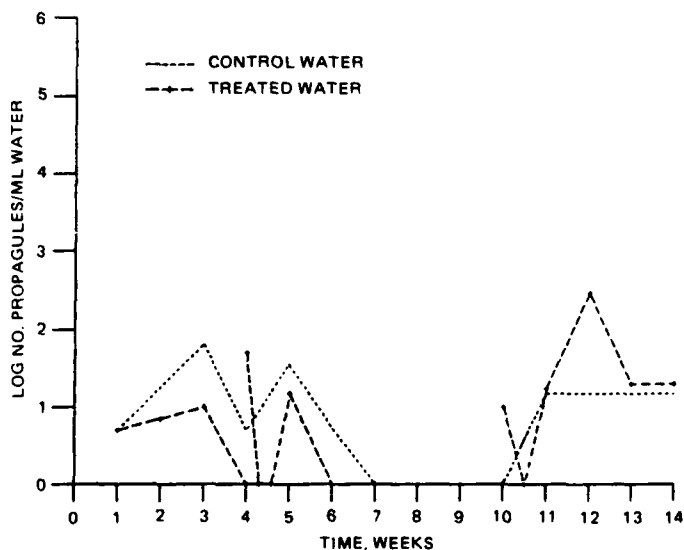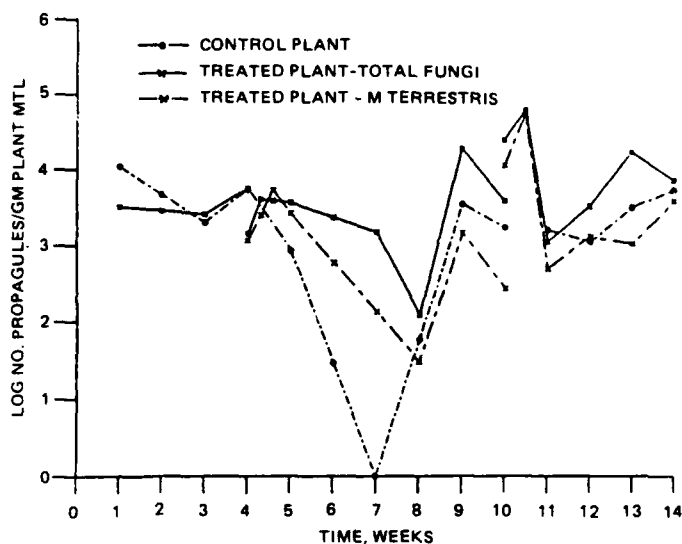

Figure 9. Fungal populations recovered on MA medium from water profiles of control and treated plots of *M. spicatum* in field experiment

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employed. The differences in the fungal counts taken from the control and from treated plant tissues were minimal, except during the sixth and seventh weeks when a significant drop in fungal numbers was observed in control samples, and on the tenth week after the second inoculation when the treated samples showed approximately a 10-fold increase in fungal propagules over the control tissues. *M. terrestris* was recovered from the treated samples following the first inoculation and throughout the course of the experiment.

Specificity testing

The responses of a number of terrestial plants to infection by *M. terrestris* are shown in Table 2. Even in those plants that were previously identified as hosts of *M. terrestris*, a high level of inoculum (2.1×10^5 CFU/ml) was required to elicit significant symptoms of infection. While the seedling stage of two species of clover, three varieties of alfalfa and one variety of bluegrass were sensitive to infection by *M. terrestris*, their mature counterparts showed no significant difference between treated and control plants. In contrast, seedlings of oats and two other varieties of bluegrass showed a higher level of tolerance to infection than did their mature stages. No significant effect was observed on soybean seedlings or mature plants.

Table 2
Infection Specificity of *Mycoleptodiscus terrestris*

Host Plant*	p-Value**	
	Seedling	Mature
Red clover	0.0000‡	0.6780
White clover	0.0000‡	0.6622
Soybean	0.2090	1.0000
Oats	0.9433	0.0152‡
Bluegrass var. *Baron*	0.0006‡	1.0000
Bluegrass var. *Kentucky*	0.3180	0.0044‡
Bluegrass var. *Merion*	1.0000	0.0000‡
Alfalfa var. *Iroquois*	0.0000‡	1.0000
Alfalfa var. *Vernell*	0.0000‡	0.3977
Alfalfa var. *Saranac*	0.0000‡	0.4747

* Previously reported as a host plant of *M. terrestris*.

** Derived from a comparison of percent mortality between treated and control plants after 8 weeks using BMDP statistical package, University of California (1985) ($p \leq 0.05$, significant; $p \leq 0.01$, highly significant).

Phosphorus analysis

The phosphorus level detected in Stockbridge Bowl exceeded 10 ppb prior to the time of the first inoculation (Figure 10). This level of phosphorus remained constant for 2 more weeks in the control plot, while in the treated quadrant, a higher level was observed. However, after the second inoculation the phosphorus level in the treated quadrant dropped from 1,200 ppb to 5 ppb within a period of 1 week and remained low until the termination of the experiment. In the control quadrant, on the other hand, the level of phosphorus after having fallen to as low as 4 ppb increased during the latter part of the experiment to 10 ppb.
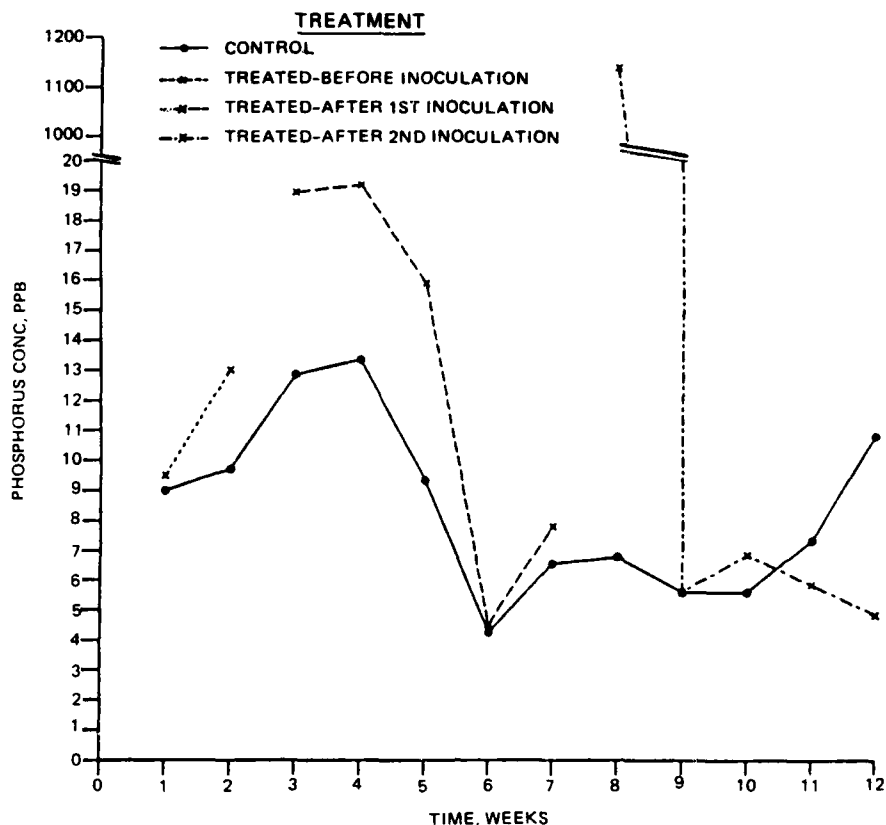
Figure 10. Phosphorus concentrations in water profiles from control and
treated plots of *M. spicatum*

DISCUSSION

The most significant aspect of the work reported here was the virtually complete decline of the milfoil in the treated quadrant (Figure 11), while in the control quadrant typical seasonal growth was maintained (Figure 12). Although results previously reported (Gunner, Limpa-amara, and Weilerstein 1985) described the decline of the treated plants by visual criteria, no significant difference in biomass was obtained. In the work reported here the biomass differences were dramatic, reflecting, it seems, not only the lethal potential of the inoculum but also the improved methodology and the effective sequestering of the respective test quadrants. In this instance, there was very little possibility that an untreated plant could drift into the treated quadrant and confound the biomass determination. The 25-fold increase in test plot size also prevented the intrusion into the treated plot by new shoots generated from adjacent root systems. In addition it appeared that the dieback generated by the treatment was much less readily obscured by the regeneration of tissue at the margins of the larger quadrant.

The 10-fold reduction in root biomass observed may, in fact, be less than actually achieved since residual roots had to be pulled up from sediment by scuba diving. Regardless of the limits of this harvesting technique, the effective decline in the root tissue is evident. With respect to the decline of the stem-leaf tissue, the loss

Figure 11. Treated plot of *M. spicatum* at termination of experiment with virtual absence of plant growth

Figure 12. Control plot of *M. spicatum* at termination of experiment with typical profusion of plant growth

due to microbial intervention is no less evident. The ratio of stem-leaf to root tissue is almost 2:1 for untreated to treated systems. The significant decline of the plant, whether measured by residual stem-leaf or by root tissues as a consequence of treatment by the microbial regimen employed confirms that this technique is an effective agent for the control of Eurasian milfoil.

Data on microbial population dynamics on the milfoil tissues and in the water profile confirmed previous observations (Gunner, Limpa-amara, and Weilerstein 1985). Thus, microbial populations are consistently higher on the plant tissues, reflecting the richer nutritional base provided by the plant. Too, the distribution of bacteria on the plant appeared sequentially determined, as evidenced by the appearance of increased numbers of the strongly pectinolytic organisms, once deterioration in plant viability was initiated by the inoculum and additional pectin residues made available. A further similarity is seen in the relatively smaller number of fungal propagules recovered from the water profile than from the plant surface. The fungal inoculum was present in all readings from the plant surface, reflecting its affinity for the plant.

A striking discrepancy exists between the impact on the plants of the first inoculum and the second inoculum. In the first instance, the inoculum was distributed by a conventional air pressure sprayer used for chemical applications. The pressure exerted at the nozzle discharge point was 200 psi and clearly, from the numbers of viable organisms recaptured at application time, there was significant mortality due to shearing. The second application, through a manifold, at a pressure of ca. 5 psi resulted in 2 to 3 orders of magnitude increase in viable cells at the time of inoculation. Ultimately, the first inoculum appeared to be below the infection threshold necessary, while the second proved adequate to initiate plant decline.

The inordinately higher water level of Stockbridge Bowl in the summer of 1986 appeared to provoke some changes in microbial population dynamics. Most noticeable was the relative constancy in microbial numbers found in the water profile without the oscillations observed in the previous season, though microbial populations on the plant surface were consistent with those previously observed. This may reflect the larger dilution effect in the water profile while the plant populations remain site-bound and nutrient determined.

Earlier studies or the specificity of *M. terrestris* (Gunner, Limpa-amara, and Weilerstein 1985) conducted with several varieties of aquatic and terrestial plants showed no evidence of infectivity. In our most recent study, plants were selected on the basis of their record of susceptibility to *M. terrestris* infection. Our results indicate that at worst *M. terrestris* is a very weak pathogen to only two of the species of clover (*Trifolium* sp.) tested. Since an inordinately high inoculum and the direct physical application by brush was necessary, it would suggest that such infection is not readily achieved. Thus, it would seem that the limited potential of *M. terrestris* as a plant pathogenic agent would not be a factor in preventing its application in an aquatic system.

Observations of the phosphorus regime during the current experimental season seem to reflect variables introduced into the environment by the early mechanical

harvesting of milfoil followed by an unusual algal bloom. Our phosphorus measurements suggest that the early cutting of milfoil, while the roots were still vigorous, allowed them to pump phosphorus from the sediment into the water profile. The release of phosphorus in turn stimulated the algal bloom. Conversely, the destruction of the plant, including its roots, by microbial means prevented phosphorus release, as evidenced by the decline of phosphorus in the treeated quadrant. It could be inferred, therefore, that under certain conditions mechanical harvesting causes an increased phosphorus release with its attendant train of deleterious events while microbiological control eliminates this potential.

CONCLUSIONS AND RECOMMENDATIONS

The results of this, our second year of field trials, confirm that *M. spicatum* may be controlled in nature by the application of watermilfoil-derived organisms *M. terrestris* and bacterium P-8. No significant residual increase in the numbers of the effective organisms was observed that would suggest perturbations in the aquatic ecosystem. The very low pathogenic potential of *M. terrestris* to a number of terrestial plants previously identified as hosts to this fungus would not appear to prevent its use in an aquatic setting.

Though the foundations for a control strategy have now been established, these must now be secured with the following:

a. The elaboration of the precise mechanism by which plant decline is achieved in response to the microbial affiliation.

b. The broadening of the scale of field testing and the fabrication of the appropriate delivery system.

c. The further testing of potential host plants to ascertain the safety of nontarget species.

ACKNOWLEDGMENTS

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Survey of the Continental United States for Pathogens of Eurasian Watermilfoil

by
William C. Zattau*

INTRODUCTION

Eurasian Watermilfoil (*Myriophyllum spicatum* L.) is an exotic, aggressive, submersed perennial aquatic plant which causes many aquatic system management problems. Conventional methods for control and management of populations of this noxious plant include herbicide treatment, mechanical harvesting, and drawdown.

Biological control of Eurasian watermilfoil has not, to date, been a viable option for use because an appropriate organism has not been available. Studies of the triploid grass carp indicate that, although the fish can be used to control Eurasian watermilfoil, it is considered undesirable for this use because of its lack of specificity for Eurasian watermilfoil and the possible indirect effects on sport fisheries. Surveys for, and studies of, insects as biological control agents of Eurasian watermilfoil have not produced suitable species (Buckingham, Bennett, and Ross 1981; Balciunas 1982; Habeck 1983).

Interest in pathogens of Eurasian watermilfoil was stimulated in the late 1960's with the Lake Venice and Northeast "disease" outbreaks (Bayley 1971). Although never proven to be the result of plant pathogen activity, the occurrences initiated research into Eurasian watermilfoil population declines (Elser 1967, Carpenter 1980, Davis and Brinson 1983, Nichols and Shaw 1986) and, likewise, interest in the use of pathogens for biological control of the species (Hayslip and Zettler 1973; Joyner and Freeman 1973; Andrews and Hecht 1981; Andrews, Hecht, and Bashirian 1982; Gunner 1983; Zattau 1985). Gunner, Limpa-amara, and Weilerstein (1985) reported results which demonstrated the ability of biological agents derived from the plant ecosystem to control Eurasian watermilfoil under simulated field conditions. Elsewhere in the proceedings, Gunner reports on a recent small-scale field demonstration utilizing microorganisms as biological control agents of Eurasian watermilfoil.

In recent years, unexplained diebacks of Eurasian watermilfoil have occurred in areas where the plant has been a problem for decades. These areas of dieback may indicate the presence of biological control agents. Although it is not likely that there are widespread native pathogen populations acting to halt the spread of Eurasian watermilfoil in the continental United States, a localized virulent bacterial or fungal pathogen population may exist. If found, such a population may be suitable as biological control agents. Therefore this thorough survey for pathogens was conducted.

* US Army Engineer Waterways Experiment Station, Vicksburg, Mississippi.

OBJECTIVES

The objectives of this study were to:

a. Examine populations of Eurasian watermilfoil in the continental United States for evidence of plant pathogen activity.

b. Isolate microorganisms from diseased tissue.

c. Select candidate microorganisms by assaying isolates for production of cellulase and pectinase, enzymes lytic to certain plant tissue.

d. Test selected candidate microorganisms for their ability to infect and damage healthy Eurasian watermilfoil plants.

MATERIALS AND METHODS

Sites

Sample sites were located in approximately 50 bodies of water located in 10 states (Figure 1, Table 1). The sites encompassed a representative cross-section of aquatic habitats with a variety of plant populations, diversity of climate, water use regimes, and water qualities.

Sample collection

Sites were scanned for diseased Eurasian watermilfoil plants, which were collected, and shipped by overnight air to WES for inspection. When no obviously
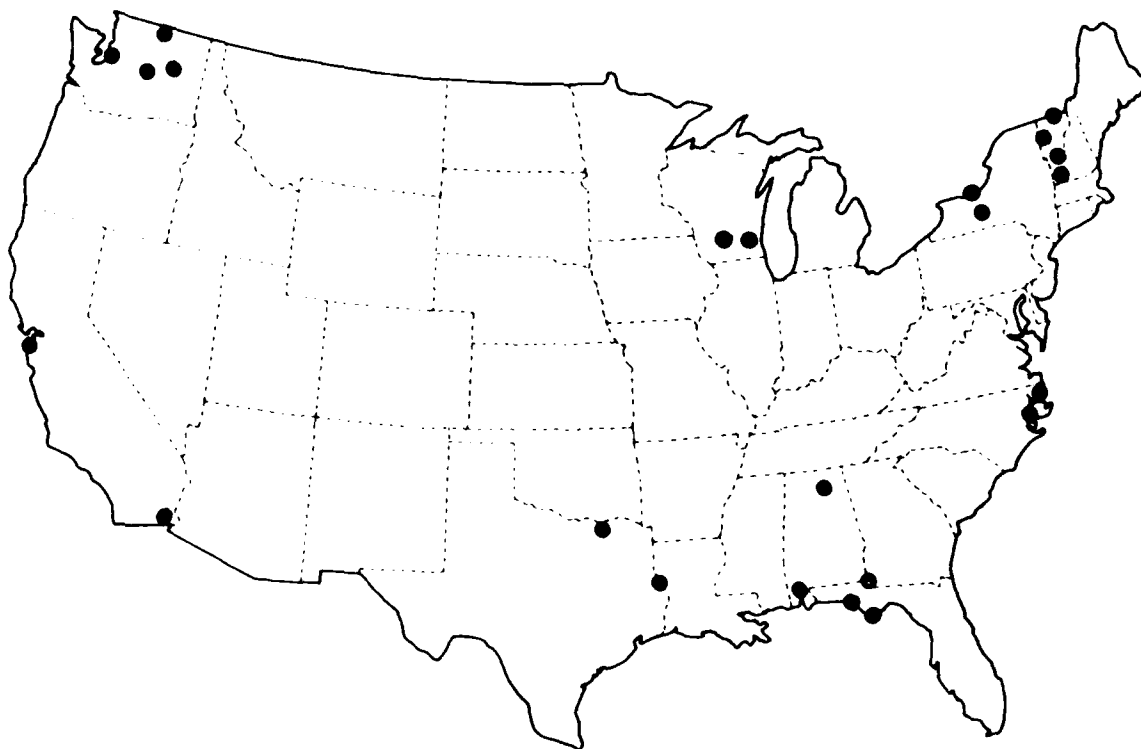

Figure 1. Sampling sites for pathogens of Eurasian watermilfoil

Table 1
Water Bodies Sampled

Northeast	Southeast
Cayuga Lake (N.Y.)	Guntersville Reservoir, (Ala).
Cornell Ponds (N.Y.)	Mobile Bay (Ala.)
Sodus Bay, Lake Ontario (N.Y.)	
	Apalachiocola (Fla).
Lake Bomoseen (Vt.)	Deer Point Lake (Fla.)
Lake Carmi (Vt.)	Lake Seminole (Fla.)
Lake Glen (Vt.)	Waukulla River (Fla.)
Lake Hortonia (Vt.)	
Lake St. Catherine (Vt.)	Coinjock Bay (N.C.
Metcalf Pond (Vt.)	Currituck Sound (N.C.)
St. Albans, Bay	Kitty Hawk Bay (N.C.)
Lake Champlain (Vt.)	Pamlico River (N.C.)
	Toledo Bend Reservoir (La.)

Northwest	Southwest
Banks Lake (Wash.)	
Lake Osoyoos (Wash.)	Pat Mayse Reservoir (Tex.)
Lake Washington (Wash.)	
Rock Island Reservoir (Wash.)	Imperial Valley Irrigation
Rocky Reach Reservoir (Wash.)	District (Calif.)
Wells Reservoir (Wash.)	Lower Crystal Springs
	Reservoir (Calif.)
	Pelarcitos Reservoir (Calif.)
	San Andreas Reservoir (Calif.)

Midwest	
Lac La Belle (Wis.)	Lake Waubesa (Wis.)
Lake Fowler (Wis.)	Lake Wingra (Wis.)
Lake Kegonsa (Wis.)	Lottes Lane (Wis.)
Lake Lilly (Wis.)	Lower Phantom Lake (Wis.)
Lake Mendota (Wis.)	Ottawa Lake (Wis.)
Lake Oconomowoc (Wis.)	Pine Lake (Wis.)
Lake Pewaukee (Wis.)	Whitewater Lake (Wis.)

diseased plants were found, plant samples were randomly collected, returned to WES as described above, and closely inspected for lesions or other microscopic disease symptoms.

Isolation techniques

When returned to the laboratory, plant material was washed in sterile water and inspected for evidence of pathogen activity. Diseased tissue was surface sterilized, cut from the stem or leaf with a sterile scalpel, and small sections of the tissue were aseptically plated on microbiological growth mediums selective for either bacteria (nutrient agar) or fungi (potato dextrose agar). Developing colonies were subcultured onto fresh plates of the appropriate medium until pure cultures were obtained.

Lytic enzyme screening

All isolates were screened for lytic enzyme production by challenging the organism with the proper assay medium. Cellulase production was determined by inoculating the isolate onto agar petri plates incorporating cellulose as the sole carbon source; pectinase production was determined by growing the isolate on

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a nutrient agar overlaid with a layer of pectate gel. A clearing of the cloudy cellulose medium indicated cellulase production by the isolate; production of pectinase was indicated by depressions or pits in the pectate gel caused by the microorganism. Isolates which tested positive for lytic enzyme production were considered candidates for further evaluation as potential biological control agents of Eurasian watermilfoil.

Test tube assay

Candidate isolates were tested for their ability to infect and damage healthy sprigs of Eurasian watermilfoil. Bacterial and fungal inoculum and a control (sterile distilled water) were introduced into test tubes containing healthy plant sprigs in a modified Hoaglands solution. A damage index value of 1 to 5 (1 = healthy, 5 = obviously dead) was assigned to each of the five replicate test tubes of each isolate. The assay ran for 6 weeks.

RESULTS

During the survey, three sites with atypical Eurasian watermilfoil plants were found: Coinjock Bay, North Carolina; Lake Bomoseen, Vermont; and Lake Osoyoos, Washington. Plants in a population of Eurasian watermilfoil in Coinjock Bay appeared diseased. These plants, collected in June 1984, were decomposing or in a state similar to advanced senescence, although all surrounding populations were healthy. Two isolates from these plants produced a significantly high (p = 0.5) mean damage index (MDI) in the test tube assay.

Plants at the Lake Bomoseen site, sampled August 1985, were covered with a dense white flocculant which, under microscopic examination, appeared as a collection of epiphytes. This covering apparently caused a large mat of Eurasian watermilfoil to exhibit symptoms of chlorosis, and gaps existed in the mat of this local population. Isolates obtained from this site did not produce a significantly high MDI.

At several Lake Osoyoos sites samples in August 1985, Eurasian watermilfoil plants were prostrate, exhibiting limited chlorosis. Microscopic examination indicated no overt pathological symptoms, and isolates from this tissue did not produce a significantly high MDI.

Although no widespread disease outbreaks were observed during this survey, many sampled Eurasian watermilfoil plants exhibited limited symptoms of phytopathogen activity such as leaf spots, stem spots, and isolated chlorosis. These plants, which were returned to WES for examination, yielded the majority of the 792 isolates collected during the survey. This collection consisted of 462 bacterial isolates and 330 fungal isolates which were maintained in pure culture. Several isolates were duplicates but were tested because they may have represented different strains of the same species.

Lytic enzyme assay of the isolates indicated that 14 bacteria and 22 fungi produced lytic enzymes. These 36 isolates were tested in the test tube assay. Five fungal isolates had a significantly greater (p = 0.05) MDI value than did the no-

treatment control at the conclusion of the 6-week test tube assay; none of the bacterial isolates had a significantly greater MDI than the no-treatment control after 6 weeks.

DISCUSSION

The fact that only three atypical Eurasian watermilfoil populations were located during the two year survey was somewhat surprising. The sample sites, which represented a geographic and climatic cross-section of aquatic systems in the continental United States including ponds, lakes, reservoirs, rivers, and canals, had resident Eurasian watermilfoil populations that were exposed to a variety of stress factors (injuries due to insect feeding, mechanical injury, physiological stress, management practices). These factors would likely predispose the plants to phytopathogen activity if the appropriate microorganisms had been present. In addition to the sample dates, these areas were under scrutiny by aquatic plant management professionals for unusual Eurasian watermilfoil diebacks. None were reported.

This survey addressed a question which many aquatic plant professionals had been posing for many years; are there native plant pathogens acting to control the spread of Eurasian watermilfoil in the continental United States? Results of this survey suggest that, during the time frame of this survey, there was no significant pathogen activity in regard to Eurasian watermilfoil in the survey areas.

CONCLUSIONS AND RECOMMENDATIONS

The conclusions of this study are:

a. No widespread disease outbreaks of Eurasian watermilfoil occurred in the sample locations during the survey period, although numerous plants evidenced limited bacterial and fungal activity.

b. Laboratory isolation procedures yielded 792 pure culture isolates; 462 were bacteria and 330 were fungi.

c. Lytic enzyme assays indicated that 36 of the isolates were candidates for test tube assays against healthy Eurasian watermilfoil plants.

d. Five fungal isolate treatments caused significantly greater damage to the plants than did the control; no bacterial isolates caused this significant damage.

It is recommended that these five fungal isolates be tested in aquarium studies to determine their efficacy as biological control agents of Eurasian watermilfoil.

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Literature Review on Senescence as an Important Factor Determining the Relationship Between Aquatic Weeds and Their Epiphytes and Pathogens

by
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INTRODUCTION

This paper summarizes the results of a review of available literature on senescence of aquatic macrophytes and microorganisms involved in senescence processes.

Some problems with aquatic weeds existed centuries ago. However, the major invasions and spreading of nuisance aquatic plants, from tropical to temperate areas around the world have occurred in this century. The primary reasons for this are increased water eutrophication and the introduction of alien species into new areas.

For centuries people tried to control aquatic weeds manually. With the development and registration of herbicides in the 1950's and 1960's, chemical control replaced most of the manual methods. However, application of herbicides has not always been the solution, and the use of chemical compounds in water is becoming more stictly regulated because of the possible side effects and potential ground-water contamination. Unsatisfactory results with chemical aquatic weed control and the increasing costs of herbicides have stimulated search for new, alternative methods.

There have been many attempts to regulate and restrict nuisance weed growth using either a selective biological agent (pathogenic bacteria and fungi, viruses, nematodes, insects, allelopathic plants) or herbivorous organisms (mostly fishes). Several large-scale research programs on biological control of aquatic weeds were started in the early seventies. Many of them have been successful, for example, the spore suspension of *Cercospora rodmanii* used for control of waterhyacinth. Many other projects that looked very promising at the stage of laboratory tests, failed in large-scale field trials.

In the last few years, integrated management of aquatic weeds, which combines several control methods, has been stressed. For example, the combination of insects (waterhyacinth weevils and waterhyacinth moth) with the parasitic fungus (*Cercospora rodmanii*) was used for controlling waterhyacinth in Louisiana. Recently, Charudattan (1986) described anintegrated control of waterhyacinth using a pathogen, insects, and sublethal rates of herbicide.

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Dramatic changes have been observed in aquatic plant populations in the last several decades. New, introduced species have become established, while others have disappeared. Increasingly disturbed aquatic ecosystems are more susceptible to sudden invasions. Introduced species often thrive at first, being free of natural parasites and foraging animals from their native locations. But often a sudden outburst of growth is, after several years, followed by an almost equally sudden decline of most of the population. Examples include a rapid spread of *Salvinia* in Lake Kariba, followed by its degeneration; expansion of *Myriophyllum spicatum* in Chesapeake Bay in the late fifties to early sixties, and its decline in the mid-seventies; and similar situations in lakes in Wisconsin a few years later.

Declines have occurred not only in populations of introduced aquatic macrophytes, but in native species as well. In the 1930's for example, an epidemic of the so-called "wasting disease" almost destroyed the normally dense beds of marine eelgrass, *Zostera marina*, along the Atlantic coast of North America and Europe. Motta (1978) described a considerable decline of *Potamogeton perfoliatus* and *Ruppia maritima* in Chesepeake Bay. *Myriophyllum spicatum* and *Potamogeton perfoliatus* stands are decreasing drastically in Neusiedlersee in Austria (Schiemer and Prosser 1976). None of these declines has a simple cause, and none has been adequately explained. Decline usually results from a combination of several interacting factors.

Throughout their life cycle, plants exhibit different sensitivity to environmental factors and are capable of a range of responses to environmental fluctuations. Different stages of plant development and different physiological processes may have different temperature and light optima and may require different photoperiod. Also, the sensitivity to infection by pathogenic fungi or bacteria is not the same throughout the plant's life cycle. Therefore, an understanding of the autecology and physiology of problematic aquatic species is necessary for successful management.

A great deal of information on physiological ecology of aquatic plants already exists. Some of the papers refer to new management possibilities using photoinhibition of reproductive organ formation. (Spencer and Anderson 1986) or naturally occurring competitive plants (Yeo and Thurston 1984). Anderson (1986) outlines future approaches and principles in aquatic plant management, based mainly on manipulation of external factors to disrupt the life cycle of a plant.

INTERACTIONS BETWEEN EPIPHYTIC MICROORGANISMS AND THEIR MACROPHYTE HOST

Microorganisms play a very important role in aquatic ecosystems. This review focuses on those aquatic microorganisms that are attached to or growing on aquatic plants and are capable of damaging them either indirectly (light limitation, physical damage) or directly (pathogens).

Many aquatic bacteria, fungi, or unicellular algae live within or on the surface of higher aquatic plants. These attached microorganisms are called "epiphytic" (epiphytes, epiphyton, periphyton). They may use plants just as physical support,

or they may be "commensals" and derive all or some of their food from the food or metabolic products of their hosts. Generally, this involves no advantages or substantial disadvantages for their hosts. Other microorganisms live as parasites and feed on the cells of their host, often producing toxic substances, and thereby causing disease and often death. These are pathogenic microorganisms.

The distinction between commensalistic and parasitic microorganisms is sometimes unclear. Often fungi or bacteria may be present on a macrophyte for a long time without any symptoms and then become parasitic when the macrophyte is stressed and weakened for any reason, e.g., when it is in a senescent stage. The term "opportunistic" is often used to describe this behavior.

The term "saprophyte" is usually meant as an opposite to parasite; however, because saprophyte refers strictly to organisms that obtain nutrients from **dead** organic material, one should use the term commensal for those epiphytic microorganisms feeding on dissolved organic matter excreted by their **living** hosts.

Microbial epiphytes have a variety of physiological relationships with their host plants. Besides being able to become pathogenic themselves, they can also reduce the inoculum potential of a pathogen either by simply occupying the area invaded by the pathogen or by producing antibiotics against the pathogen.

Very little information is available on the physiology, nutrition, energetics, or pathogenicity of epiphytes in an aquatic ecosystem and the actual interactions between macrophyte and attached microorganisms. Some macrophytes do not have periphyton due to the release of compounds toxic to algae. Usually, however, dense and productive periphyton communities of algae and bacteria develop on most macrophytes, often embedded in a carbonate muco-organic complex. Shading and nutrient depletion at the leaf surface are the major effects on the macrophytes. Heteroptrophic microorganisms and algae can utilize dissolved organic carbon released by the macrophyte. The metabolic activity of the heterotrophs, in turn, promotes the growth of algae, and probably of the macrophyte, by increasing the rate of mineral cycling. Figure 1 shows interactions between macrophytes and their epiphytes.
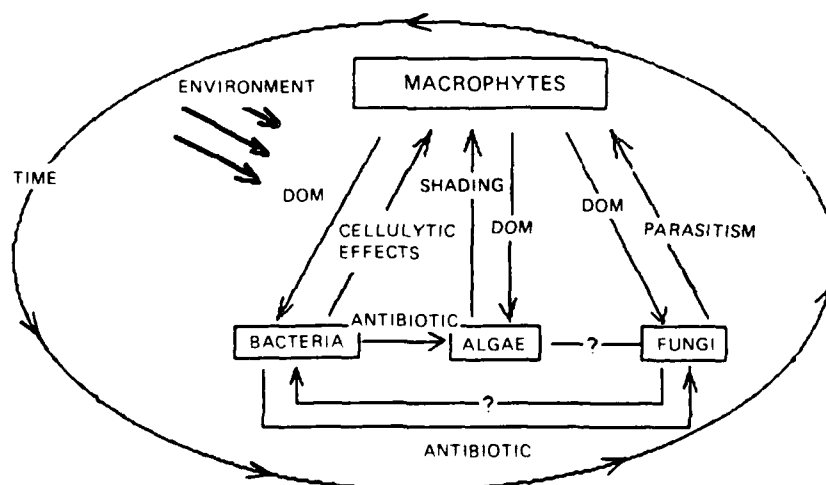

Figure 1. Generalized pattern of interactions in macrophyte-epiphyte microflora complex (DOM = dissolved organic matter)

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SENESCENCE

Death of an organ or of whole plants is always preceded by the process of senescence, which may be regarded as the final phase in development that leads to cellular breakdown and death. More precisely, in leaves, it relates to the loss of structural integrity and photosynthetic competence in the chloroplast. Of the steps in biological development, senescence is one of the least defined. Changes in growth rates and vigor and increases in susceptibility to environmental stress or pathogens are generally connected with senescence. With a few exceptions, we do not know much about what factors influence or trigger senescence in aquatic macrophytes. There are reports indicating that senescent plants are probably most susceptible to infections by pathogens.

A distinction can be made between organ senescence and whole plant senescence. In most plants each leaf has only a limited life span so that, as the shoot continues to grow in height, the older leaves at the base tend to senesce and die. This pattern of senescence has been described as sequential senescence. Figure 2 shows parts of a plant that can undergo senescence.
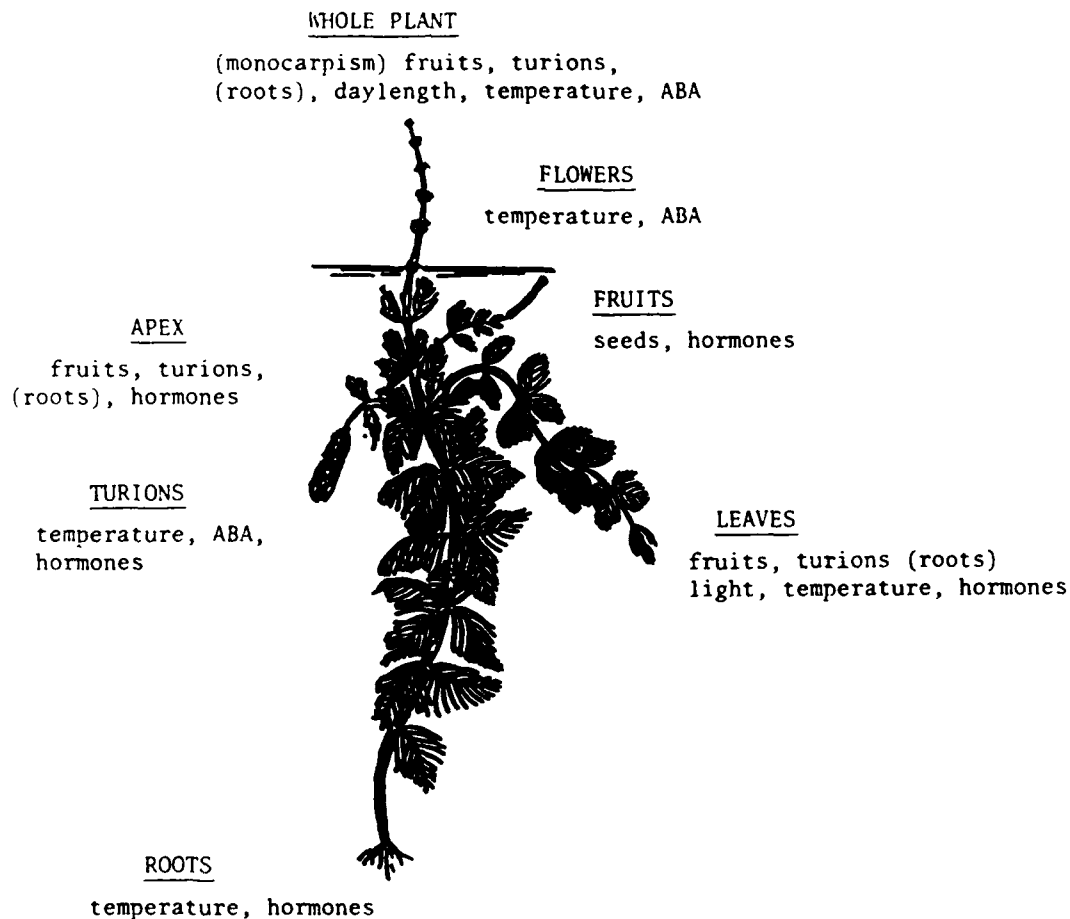

Figure 2. Parts of a submersed aquatic plant that can undergo senescence. Environmental and hormonal influences are indicated together with those parts of the plant that can exhibit correlative controls (ABA = abscisic acid)

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Disease is often regarded as a cause of senescence. No matter how well the undamaged parts of an infected plant compensate for the local effects of a pathogen, eventually the physiological control systems fail to function properly, and senescence and decay take over. Very little is known about the nature of hormonal changes in diseased plants. In many cases it is not known whether hormonal imbalances in diseased tissues are the cause or the result of infection. In other words: Does the plant senesce because it was attacked by the pathogen, or was it attacked by the pathogen because it was already in a senescent stage and therefore more susceptible to pathogen attack?

There is a general belief that weak pathogens are able to advance senescence. The experimental evidence for this phenomenon is rather conflicting. In many cases senescence in infected plants is accelerated by the reduction in the rate of photosynthesis brought about by toxic substances produced by the pathogen. There are several ways by which plant pathogens exert stress or influence on their host plants. They can produce enzymes that dissolve cell walls (cellulolytic, pectolytic activities), surface-bound and extracellular polysaccharides, toxins, or phytohormones. Very few papers deal with aquatic plant pathogens.

It is clear that although a number of interesting approaches to the problem of senescence in plants are being taken, it is too early to be able to present a single, overall hypothesis to account for all the facts. However, in general we can recognize that the initiation of senescence involves a change in the relative levels of growth hormones, and this change in hormonal status may be caused either by internal factors or environmental stimuli.

There are apparently two types of senescence. One is the senescence that proceeds as a natural part of the whole plant development and is primarily controlled by internal genetic and correlative factors. The other one can be called enforced or induced senescence and reflects the changes caused by unfavorable environmental factors (mineral deficiencies, insufficient light) or diseases. We are mainly interested in the latter type of senescence and its possible manipulation. Why do we want to manipulate senescence? There are potential benefits to be gained in terms of plant productivity (crop plants) if the senescence is delayed. For aquatic weed problems, however, we would like to accelerate senescence (selectively, if possible) and make the weeds more susceptible to disease.

Little information has been published on senescence in aquatic plants, except for papers by Jana and Choudhuri (1982) and Kar and Choudhuri (1985) describing in detail senescence in both intact and detached leaves of the submersed macrophytes *Vallisneria spiralis*, *Hydrilla verticillata*, and *Potamogeton pectinatus*. Senescence is briefly mentioned in several production-ecological studies, and some data exist on translocation of minerals before and during senescence.

SENESCENCE AND PATHOGEN INTERACTIONS: ADVANTAGES AND LIMITATIONS

Many data exist supporting the idea that senescent plants are more susceptible to pathogen infection than nonsenescent plants. We do not know the exact

mechanism responsible for this phenomenon. There is probably more than one reason but generally a senescent plant is weakened and less resistant than a nonsenescent one.

Senescence proceeds as a natural part of plant development, controlled primarily by internal genetic and correlative factors. But senescence can also be induced by unfavorable environmental factors. Table 1 summarizes the possible means of senescence induction and indicates which of them might be used to manipulate senescence of undesirable aquatic weeds in the field.

Environmental factors do not always induce senescence in the whole population of a plant species. Only a few less vigorous plants may become senescent and die, while the rest continue growing. The application of a pathogen at the proper moment, i.e., when the population is partially weakened, may greatly enhance its spread. The scheme in Figure 3 shows the possible interactions of induced senescence with pathogens. Nothing, so far, is known about the possibility to induce pathogenicity in opportunistic microorganisms which would then attack plants and cause their senescence and, eventually, death.

Table 1
Senescence-Inducing Factors

Inducing Factor	Feasibility Rating*
Low light	++
Nutrient availability	+
Photoperiod	++
Temperature	+
Dense periphyton	+
External growth regulators (ABA)	++
Sublethal herbicide doses	+++
Herbivores	++

* Rating symbols are:
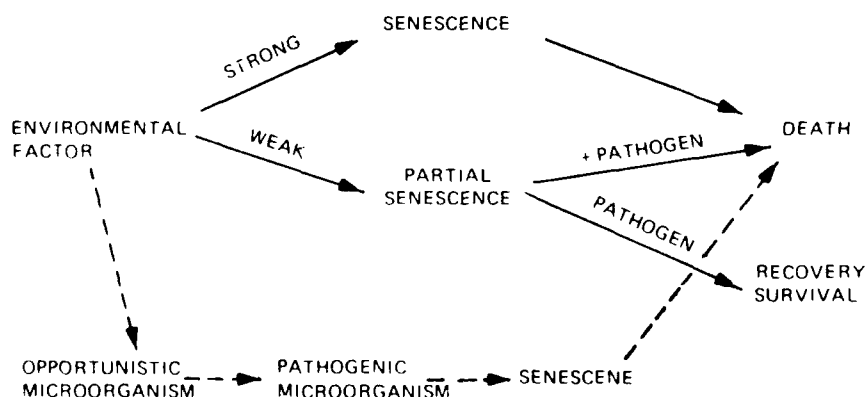 + = infeasible, ++ = moderately feasible, +++ = highly feasible.

Figure 3. Scheme of interactions between environmentally induced senescence and pathogens

More research is needed before the combined effect of senescence and pathogens can be practiced. It will be necessary to find the most practical factor for inducing senescence in a particular species, the most effective type of pathogen, at which stage of senescence to apply a pathogen, etc. The limitations include the varying response of a plant species to the factor inducing senescence, unpredictable interactions of a pathogen with another microorganisms present in water, and development of plant resistance to pathogens.

CONCLUSIONS

Senescence

The main facts regarding senescence are as follows:

- Senescence is an important but not very well understood stage in a plant's life cycle. It is controlled by internal and external (environmental) factors. The precise mechanism of senescence induction is not known. However, in general, the initiation of senescence involves an imbalance in the relative levels of growth hormones, and the change in hormone status can be caused by internal factors or environmental stimuli.

- The following environmental stimuli were reported to be involved in senescence initiation: light, daylength, temperature, mineral nutrients, and pathogens. When pathogens attack plants, it is not always known whether hormonal imbalances are the cause or the result of infection.

- Senescence is accompanied by the change in enzyme activities. Peroxidases, glutamate dehydrogenase, endopeptidases, and some hydrolases have been reported to increase their activities during senescence. Dehydrogenases, ribulosebiphosphate carboxylase, glutamine synthetase, and glutamate synthase generally decrease their activities during senescence.

- An extensive literature search identifies cytokinins as the most generally effective senescence-retarding growth regulators. Abscisic acid (ABA) and ethylene, on the other hand, are known as promoters of senescence. In many cases, cytokinins and ABA interact in a competitive manner.

- Decreases in chlorophyll content and protein amount are most often measured as senescence characteristics. Senescence may start long before changes in these parameters become apparent. No universal marker of senescence has been found thus far.

Microorganisms on aquatic plants

Our review of microorganisms on aquatic plants has concluded the following:

- Aquatic macrophytes often serve as a substratum for epiphytic microorganisms, namely algae, bacteria, and fungi. The amount and diversity of epiphytic microorganisms generally increase with the age of their host. The main reason is the increasing amount of organic matter excreted by the aging host plant.

- Microorganisms live mostly as commensals (saprophytes) on their hosts but may become parasitic as the hosts become stressed and/or approach senescence.

- Since submersed aquatic plants usually have very thin and reduced cuticle, bacteria do not need wounds for entry into plants. Bacteria often invade and degrade the epidermal cell walls. Some are known to produce lytic enzymes.

Some bacteria were reported as being able to produce antibiotics.

- Fungi are usually regarded as the main decomposers of dead organic matter in aquatic ecosystems. Many species are known to produce enzymes capable of degrading cellolose, pectin, starch, etc. Besides enzymes, some fungi also produce toxins and antibiotics. The phytotoxicity of fungi has a potential use as bioherbicide. In our search, we found 150 fungal species reported as potential parasites on aquatic plants.

- Epiphytic algae do not usually cause any harm to their host plants unless the colonization is so dense that it shades plants or, especially in combination with inorganic silt particles, becomes so heavy that the host plants are damaged and dragged to the bottom.

- Few viruses were reported from aquatic plants. This may have potential for biological control, but the data at this point are sufficient to consider this alternative.

- Nematodes are known to contribute to macrophyte degradation in certain cases. They wound the tissue, allowing an entry for infection. So far, at least 32 genera of nematodes parasitic on aquatic plants have been reported.

RECOMMENDATIONS

The following research is recommended:

- Find the most efficient means of inducing senescence in aquatic weeds that can be eventually applied on a large scale.

- Evaluate the effectiveness of combining induced senescence with pathogen application.

- Study the effect of combining induced senescence with naturally occurring microorganisms (opportunitistic pathogens).

- Initiate large-scale field tests if small-scale research proves that the manipulation of senescence in aquatic weeds in combination with pathogen application is feasible.

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Use of Allelopathy for Aquatic Plant Management

by
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INTRODUCTION

Use of allelopathic terrestrial plants has received attention in agriculture as a weed management strategy. Putnam (1983) has achieved success with annual rotation of allelopathic crops or companion plantings of them with perennial crops. He has been able to suppress up to 95 percent of several important weeds. Allelopathic aquatic plants may provide a management system for undesirable aquatic vegetation. That replacement of an undesirable species by a desirable one could provide a long-term, site-specific method of aquatic plant management was suggested over 30 years ago by Osborne (1954). They observed that in a 2-year period dwarf arrowhead and needle spike rush crowded out pondweed and suggested the desirability of planting one or both plants to prevent "growth of the ranker growing pondweeds." This method of aquatic plant management is not likely to replace other control methods, either biological or chemical, but certainly it should be complementary to them. This paper presents preliminary results of a feasibility study of use of allelopathic aquatic plants for aquatic plant management.

Our plan for assessing the feasibility of use of allelopathic aquatic plants as replacement species for aquatic plant management involves the following:

- Step 1. A thorough search of the literature to compile a list of "known" alelopathic aquatic plants.
- Step 2. Selection of a list of "most likely candidates" from the list of allelopathic aquatic plants.
- Step 3. Bioassay of the selected plants to compare the relative activities of each.
- Step 4. Culture requirements of the most growth inhibitory plants of those tested in Step 3 will be determined, and from these requirements, experimental designs for future field testing will be suggested.

Step 1, the literature search, is essentially complete. About 100 papers were located, and this bibliography will be included in the project year-end report both in hard copy and on computer disk. All selected plants have been subjected to bioassay against a terrestrial target plant species, and bioassay against an aquatic plant target species is in progress.

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MATERIALS AND METHODS

Literature search

The University of Southern Mississippi's computer-assisted retrieval system (SCARS) was used to search **Chemical Abstracts, Biological Abstracts** and **Agricola** from 1970 to date. Search strategy used allelopathy or competition or toxic and aquatic plants (or weeds or vegetation). In addition, 20 specific plant names were searched.

Plant collection and processing

Sufficient quantities of each selected plant were collected by hand and transported to the laboratory in plastic bags. In those cases where the plants were kept longer than 0.5 hr after collection, they were kept on ice. The plants were washed free of debris, and spread on newspaper to dry to the "drip dry" stage. Voucher specimens were deposited in the University of Southern Mississippi herbarium. A 200-g aliquot of the drip-dried plants was thoroughly blended with 200 ml of distilled, deionized water, and the resulting pulpy mixture was refrigerated for 24 to 72 hr. The mixture was filtered through cheesecloth to remove the majority of the celluosic material, through filter paper to remove smaller particulate matter, and finally through a 0.45-μ millipore filter to sterilize the solution. This solution was either assayed immediately or stored frozen.

Lettuce seedling bioassay

Assays were performed according to Elakovich and Stevens' method (1985). Each plant extract was measured at three extract concentration levels (1, 5, and 10 ml per test plate), each diluted to 40 ml by addition of 30 ml of 0.5-percent agar and the appropriate amount of distilled water. The control contained 30 ml of 0.5-percent agar and 10 ml of distilled water.

RESULTS AND DISCUSSION

Of the approximately 100 papers located in the literature search, only 18 actually reported experimental evidence of support of allelopathic aquatic plants. Of these, five report on *Eleocharis coloradoenisis*, dwarf spikerush, as the allelopathic plant, while another four report on *Typha latifolia*, cattail. Other plants identified are listed in Table 1.

A problem with much of this literature is that definitive testing methods for allelopathy have not been developed and, consequently, some inconsistencies are found in the literature. For example, Yeo (1976) reported that *Eleocharis acicularis* and *E. coloradoensis* can encroach upon the area previously occupied by *Potamogeton crispus* and *P. pectinatus* and prevent their growth. Yeo (1980) reported *P. crispus* was not replaced by *E. coloradoensis*. Grace, in 1983, refuted McNaughton's (1968) earlier, much-quoted report of the autotoxicity of *Typha latifolia*. Some of these publications report field observations alone (Nichols and Shaw 1983), some report activities of plant extracts (Frank and Dechoretz 1980; Ashton, di Tomasi, and Anderson 1985), and others report carefully constructed

Table 1
Allelopathic Aquatic Plants

Allelopathic Plant	Affected Plant(s)	Reference
Ambrosia trifida	Lettuce, radish, tomato cucumber	Bonasera, Lynch, and Leck, 1979
Bidens laevis	Lettuce, radish, tomato, cucumber	Bonasera, Lynch, and Leck, 1979
Brasenia schreberi	Lettuce	Elekovich and Wooden, in press
Carex hudsonni	*Phragmites communis*	Szczepanska 1977
Chara vulgaris	*Vallisneria americana*	Titus and Stephens 1983
Eleocharis acicularis	Water weeds	Nichols and Shaw 1983
Eleocharis acicularis	*Elodea canadensis* *Potamogeton pectinatus* *Potamogeton crispus*	Yeo and Fisher 1970
Eleocharis coloradoensis	*Potamogeton pectinatus* *Potamogeton nodosus* *Potamogeton pusillus* *Potamogeton foliosus* *Najas quadalupensis* *Elodea canadensis* *Elodea nuttallii*	Yeo 1980
Eleocharis coloradoensis	*Potaamogeton nodosus* *Potamogeton pectinatus*	Frank and Dechoretz, 1980
Eleocharis coloradoensis	*Zannichellia palustris* *Elodea nuttallii* *Elodea canadensis* *Hydrilla verticillata* *Potamogeton nodosus* *Potamogeton pectinatus* *Myriophyllum spicatum*	*Yeo and Thurston 1984
Eleocharis coloradoensis	*Hydrilla verticillata* *Potamogeton pectinatus* Tomato cell culture Lettuce seedling roots	Ashton, de Tomasi, and Anderson, 1985
Eleocharis coloradoensis	Water weeds	Nichols and Shaw 1983
Eleocharis parvula	Water weeds	Nichols and Shaw 1983
Equisetum fluviatile	*Phragmites australis*	Szczepanski 1977
Equisetum limosum	*Phragmites communis*	Szczepanska 1971
Equisetum palustris	*Phragmites australis* *Typha latifolia*	Szczepanski 1977
Hydrilla verticillata	*Ceratophyllum demersum* *Ceratophyllum muricatum*	Kulshreshtha and Gopal 1983
Ipomoea aquatica	*Pennisetum Typhoideum*	Singhvi and Sharma 1984
Ludwigia adscendens	*Pennisetum Typhoideum*	Singhvi and Sharma 1984
Myriophyllum spicatum	*Najas marina*	Agami and Waisel 1985
Peltandra virginica	Lettuce, radish, tomato, cucumber	Bonasera, Lynch, and Leck, 1979
Phragmites australis	*Carex elata*	Szczepanski 1977
Potamogeton amplifolius	*Vallisneria americana*	Titus and Stephens 1983

(Continued)

Table 1 (Concluded)

Allelopathic Plant	Affected Plant(s)	Reference
Sagittaria graminea	*Hydrilla verticillata*	Sutton 1986
Sagittaria pygmaea	Rice	Lee and Guh 1982
Schoenoplectus lacustris	*Potamogeton australis*	Szczepanski 1977
Schoenoplectus lacustris	*Equisetum limosum*	Szczepanska 1971
Typha latifolia	*Acorus calamus* *Phragmites communis*	Szczepanski 1977
	Glyceria maxima *Phragmites australis* *Equisetum fluviatile* *Typha and angustifolia*	
Typha latifolia	Lettuce, radish, tomato, cucumber	Bonasera, Lynch, and Leck, 1979
Typha latifolia	*Typha latifolia*	McNaughton 1968
Typha latifolia	*Phragmites communis*	Szczepanska 1971

competitive studies of whole plants. It is therefore difficult to draw conclusions as to the most promising allelopathic aquatic plants from the literature available.

Many of the 22 plants identified in Table 1 as allelopathic aquatic plants are not deep-water plants. Many are shoreline plants that would be ineffective in controlling the worst aquatic weeds, *Hydrilla verticillata* (hydrilla) and *Myriophyllum spicatum* (Eurasian watermilfoil). Some are not native to the southeast United States and so were not available in this feasibility study. The 16 plants listed in Table 2 were selected as potentially useful allelopathic aquatic plants. They were selected for a variety of reasons. *Brasenia schreberi* and *Eleocharis acicularis*, for example, were selected because they had been reported as allelopathic (Table 1). *Eleocharis obtusa* was included because of the importance of *Eleocharis* in allelopathy. We were unable to include *E. coloradoensis* because it does not grow in the southeastern United States. Both hydrilla and Eurasian watermilfoil were selected so that we could evaluate their activity in our assay system. *Myriophyllum aquaticum* was selected because it is a desirable plant in the same genus as the nuisance Eurasian watermilfoil. The remaining plants were selected either because of their observed potential allelopathic activity as determined by their growth patterns, or because they are desirable replacement species.

Aqueous extracts of these 16 plants were subjected to lettuce seedling bioassay as a first "easy" assay for allelopathic potential. Advantages of this assay method are experimental simplicity, short time requirements and sensitivity. The major disadvantage, however, is that aquatic plants are being tested against a terrestrial plant target species (Ashton, de Tomasi, and Anderson 1985). Factors influencing the growth of aquatic plants may be very different from those factors influencing terrestrial plant growth. We, therefore, also plan to subject the aqueous extracts to a bioassay involving the aquatic plant *Lemna minor*. A third proposed assay will involve *Hydrilla verticillata* as the target species. This latter assay will allow the evaluation of activity toward an aquatic plant, but more importantly, toward

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hydrilla, one of the most noxious of aquatic plants. We are particularly interested in determining whether there is good correlation between results from terrestrial plant bioassay and aquatic plant bioassay.

Results from the lettuce seedling bioassay at three extract concentrations (1, 5 and 10 ml per test plate) are presented in Table 2. Extracts of six plants inhibited greater than 77 percent of lettuce seedling radical growth. These are, with the most inhibitory first, *Nymphaea odorata* roots, *Juncus repens*, *Vallisneria americana*, *Brasenia schreberi*, *Ceratophyllum demersum*, *Eleocharis acicularis* and *Nymphaea odorata* leaves and stems. Of these, *N. odorata* root extract was

Table 2
Results of Lettuce Seedling Radical Inhibition by
Aqueous Extracts of Selected Aquatic Plants

Plant	Percent of Control*,**			
	Control	1 ml	5 ml	10 ml
Brasenia schreberi Gmel.	100a (60)	107b (40)	46c (40)	18d (40)
Cabomba caroleniana Gray	100a (30)	99a (30)	51b (30)	42b (30)
Ceratophyllum demersum L.	100a (45)	34b (40)	26c (40)	20d (40)
*Eleocharis acicularis** (L.) R.&S.	100a (45)	69b (30)	39c (30)	22d (30)
*Eleocharis obtusa** (Willd) Schultes	100a (45)	73b (36)	38c (36)	25d (36)
Hydrilla verticillata (L.f.) Royle	100a (30)	91a (30)	66b (30)	39c (30)
Juncus repens Michx.	100a (60)	49b (40)	18c (40)	14c (40)
Limnobium spongia (Bosc.) Steud.	100a (30)	63b (30)	32c (30)	27c (30)
Myriophyllum aquaticum (Vell.) Verdc.	100a (40)	71b (40)	57c (40)	51c (40)
Myriophyllum spicatum L.	100a (45)	68b (40)	48c (40)	36d (40)
Najas quadalupensis (Spreng.) Magus	100a (30)	91a (30)	38b (30)	26c (30)
Nymphaea odorata Ait. (leaves and stems)	100a (60)	101a (40)	68b (40)	22c (40)
Nymphaea odorata Ait. (roots)	100a (60)	40b (40)	9.3c (40)	5c (35)
Nymphoides cordata (Eil.) Fern.	100a (60)	76b (40)	52c (40)	31d (40)
Potamogeton foliosus Raf.	100a (40)	73b (40)	43c (40)	39c (40)
Sparganium americanum‡ Nutt.	100a (60)	77b (45)	48c (45)	40d (45)
Vallisneria americana	100a (60)	113b (40)	45c (40)	17d (38)

* Values followed by the same letters are not significantly different according to the Duncan's Multiple Range Test at p ≤ 0.05.

** Values in parenthesis are numbers of cases.

† 200 g fresh plant material was blended with 350 ml water.

‡‡ 200 g fresh plant material was blended with 225 ml water.

the most active with 95-percent inhibition of lettuce radical growth by 10 ml of aqueous extract. *Ceratophyllum demersum* extracts brought about the greatest inhibition (66 percent) at the 1-ml concentration. Both *B. schreberi* and *V. americana* are strongly inhibitory at 10 ml levels, but are stimulatory at 1-ml levels. Rice (1984) has suggested that many, perhaps most, plant growth inhibitors may be growth stimulators at some much lower concentrations.

FUTURE WORK

The next step of this project is to carry out bioassay of the aqueous plant extracts with an aquatic plant as the target species. We are currently developing optimum growth conditions and axenic stock cultures of *Lemna minor*, the selected target species. Work beyond this initial year will include bioassay with *H. verticillata* as the target species as well as greenhouse studies to demonstrate allelopathic capabilities. These latter efforts will involve competitive studies that are much better than bioassays for determining allelopathic potential and are also more time and resource intensive. Field observation studies will involve travel to areas infested with either Eurasian watermilfoil or hydrilla or both and surveys of the associated vegetation. Areas that seem to be similar to these infested areas, but are free of hydrilla and Eurasian watermilfoil will also be observed. Results from this future work will lead to field trials of use of allelopathic aquatic plants as replacement species for noxious weeds in aquatic environments.

ACKNOWLEDGMENTS

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Dispersing Waterhyacinth Biocontrol Agents
in the Galveston District

by
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BACKGROUND

Major waterhyacinth infestations occurring in Texas exist in an area south of a line from Logansport to Austin, and east of a line from Austin to Brownsville. Limited efforts to establish biocontrol agents within these waterhyacinth infestations were conducted by the Texas Parks and Wildlife Department (TPWD) in the mid-1970's. In 1979, the US Army Engineer District, Galveston (SWG), requested that the US Army Engineer Waterways Experiment Station (WES) conduct field studies to evaluate the status of waterhyacinth biocontrol agent populations in the SWG and, subsequently, to establish populations of those biocontrol agents not reported in the surveys.

The initial district-wide survey was conducted by the WES in June 1980. Observations indicated that none of the biocontrol agents were present (Cofrancesco 1982). Between 1980 and 1981, WES researchers established a *Neochetina bruchi* founder colony near Wallisville, Tex. By 1982, this colony was sufficiently established to provide the source for releases at additional sites. Between 1982 and 1984, WES researchers made numerous releases of *N. bruchi* at four sites within the SWG (Cofrancesco 1984). Additionally, releases of *N. eichhorniae* and *Sameodes albiguttalis* were made at several locations.

The WES also conducted District-wide distribution surveys each year to monitor biocontrol agent dispersal. These surveys documented the continual westward dispersonal of *N. eichhorniae* into the SWG from populations in southern Louisiana. The significance of this natural dispersal by *N. eichhorniae* is realized by comparing results (Cofrancesco, in preparation) of the 1984 survey. This survey indicated that *N. eichhorniae* was established in every major waterhyacinth infestation between Port Arthur and Corpus Christi. The distribution of *N. bruchi* was limited to the immediate vicinities of the five release sites. Established colonies of *N. bruchi* could only be found at two locations near Wallisville; at sites within the J. D. Murphree Wildlife Management Area (JDM) west of Port Arthur; at sites in Hog Bayou east of Tivoli; at a site on Lake Corpus Christi near Dinero; and at two sites along the Guadalupe River east of Belmont. The only documented colony of *S. albiguttalis* was located in the JDM.

Though *N. eichhorniae* had demonstrated natural dispersal throughout the SWG between 1980 and 1984, observations indicated that this dispersal had resulted from the natural overflow from the Louisiana populations which had been

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developing since 1974. Since *N. bruchi* was not extensively established in Louisiana, it was likely that natural dispersal by this species would not attain such significance in the near future.

Similarly, accomplishments toward achieving District-wide dispersal of *S. albiguttalis* by the end of 1984 were seemingly limited to the establishment of the single colony within the JDM. However, researchers in Florida (Center 1981) and in Louisiana (Sanders and Theriot 1986) had reported that this species had often accomplished widespread dispersal before attaining easily detectable population levels in any given area. Thus, we were confident that the *S. albiguttalis* distribution in the SWG was not limited to the single location detected in the JDM.

PURPOSE AND OBJECTIVES OF FY 85 AND FY 86 EFFORTS

Purpose

After reviewing the above-stated results, the SWG requested that the WES provide continued assistance in attaining District-wide dispersal of *N. bruchi* and *S. albiguttalis*. Additionally, the SWG requested that the WES work closely with the TPWD during field activities to transfer biocontrol technology to TPWD personnel. Once achieved, this transfer of information would enable the TPWD to more successfully incorporate biocontrol technology within operational control strategies.

Objectives

Objectives of the FY 85 and FY 86 efforts were to:

- Establish new colonies of *N. bruchi* in strategic locations which would effect dispersal within connecting waterbodies plagued with significant waterhyacinth problems.
- Conduct field surveys to maintain knowledge of the current distribution of *S. albiguttalis* in the SWG.
- Demonstrate field techniques for identifying, collecting, transporting, and releasing the three biocontrol agents to TPWD personnel.

ACCOMPLISHMENTS

Establishment of *N. bruchi* Colonies

Based on information from the TPWD personnel, colonies of *N. bruchi* were established at four new sites: Houssen Bay on Toledo Bend Reservoir; Taylors Bayou, approximately 10 miles north of the JDM; Penwah Slough on Lake Livingston; and the Nueces River, in a tributary approximately 6 miles upstream of Lake Corpus Christi. These sites were selected because they contained nuissance waterhyacinth infestations which could not be easily accessed with herbicide application equipment. An additional *N. bruchi* colony was established in Osyter Creek at the request of the Brazos River Authority. The Oyster Creek site is located approximately 15 miles northeast of Sugarland, Texas.

Dispersal of *S. albiguttalis*

No *S. albiguttalis* were sighted during the 1985 surveys. In May 1986, *S. albiguttalis* were collected in Louisiana and released in the JDM and at sites near Wallisville. Subsequent to these releases, a small colony of *S. albiguttalis* was located near the *N. bruchi* release site at Oyster Creek. In July 1986, *S. albiguttalis* was observed within every waterhyacinth infestation surveyed from Port Arthur to Corpus Christi.

Demonstration of field techniques

Field techniques for identifying, collecting, transporting, and releasing both species of *Neochetina* were demonstrated to TPWD personnel on numerous occasions. Demonstrated collecting techniques different in respect to intended time (day or night) of collection. Day collecting can be accomplished by either hand-picking individuals from plants or by collecting large quantities of plants from areas with high *Neochetina* densities. Night collecting is best accomplished with sweep nets. Techniques for transporting *Neochetina* differ depending on collection techniques and length of time before the release is made. Demonstrated release techniques differed only in how the collections had been made.

Field techniques for work with *S. albiguttalis* are discussed fully by Center (1981). Techniques demonstrated to TPWD personnel were limited to visual recognition of plants infested with this biocontrol agent, and subsequent collection, transport, and release of infested plants at new locations.

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Biological Control of Waterlettuce

by
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INTRODUCTION

Waterlettuce, *Pistia stratiotes*, is a floating aquatic plant that is widely distributed throughout the tropical and subtropical areas of the world. While it is a serious problem in southeast Asia, Africa, and India (Cook et al. 1974, Holm et al. 1977), in Florida it has generally been considered a minor aquatic weed, although there is evidence that it is becoming increasingly more important. The decrease of waterhyacinth populations due to maintenance herbicide treatments and the effects of the three introduced biological control agents have left areas vulnerable for waterlettuce invasion. The latest figures (Schardt 1985) indicate that 7,349 acres of Florida waters were infested, despite treatment of an estimated 16,151 acres with herbicides during the year. Waterlettuce can be controlled with herbicides, but treatment is expensive and must be repeated.

In addition to increasing water loss through transpiration, interfering with recreation and irrigation use, and impeding water flow, waterlettuce is detrimental in another way. Larvae and pupae of the mosquito genus *Mansonia* obtain their oxygen by attaching to waterlettuce roots. Lounibus and Escher (1985) found that *Masonia dyari* and *M. titillans* comprised 95.9 percent of the nearly 46,000 specimens of mosquitoes collected in emergence traps over waterlettuce. In a waterlettuce-covered phosphate pit in Polk County, Florida, the population of *Mansonia* larvae and pupae was estimated at 30 million per acre.* *Mansonia titillans* is a ferocious biter of man, and these mosquitoes are potential transmitters of diseases.

Biological control of waterlettuce, while feasible, was not considered earlier because of the relatively minor importance of this weed compared to other aquatic weeds and the high cost of foreign exploration and other studies necessary to introduce a biological control agent. The success of the Australians in controlling waterlettuce with the weevil *Neohydronomus pulchellus* introduced from Brazil (Harley et al. 1984), the increase in waterlettuce populations, and the support and encouragement of the US Army Engineer District, Jacksonville, motivated us to attempt biological control of waterlettuce in Florida.

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RESULTS AND DISCUSSIONS

Surveys were made from July 1985 through June 1986 to determine (a) whether the weevil was in Florida and (b) what organisms were associated with waterlettuce in Florida. More than 50 populations were sampled, many of them during each season. At least 20 plants were collected from each population, and replicate samples were collected from some of the sites. Plants were returned to the laboratory and submersed under water for 4 hr. Organisms coming to the surface were collected, and after 4 hr, the plants were removed and the water was strained to collect any remaining specimens. Specimens were identified and segregated by type. Scuds, midge larvae (Chironomidae), biting midge larvae (Ceratopogonidae), soldier fly larvae (Stratiomyidae), and water beetle adults and larvae (Noteridae and Dytiscidae) were the organisms most frequently collected. These organisms apparently use the roots of waterlettuce as a shelter and have little or no effect on the plant.

The most damaging phytophagous insect was the samea caterpillar *Samea multiplicalis* whose larvae tunnel through the leaves and cause severe damage, especially in the fall. The samea caterpillar also feeds on salvinia, azolla, and occasionally on waterhyacinth (Habeck, Haag, and Buckingham 1985). Another moth closely associated with waterlettuce is *Petrophila drumalis*. This species appears to be limited to the southern half of Florida. Because the aquatic caterpillars feed on the root hairs, their presence can be discerned by the absence of lateral root hairs. A third species, the waterlily leafcutter, *Synclita obliteralis*, the most common aquatic caterpillar in Florida, was occasionally common on waterlettuce. The caterpillars live within a portable case filled with air. They never go very deep in the water and may climb out of the water to feed and to cut leaf portions for their case. This species is highly polyphagous and has been recorded from more than 40 plant species (Habeck, Haag, and Buckingham 1985). A less common but more obvious caterpillar on waterlettuce is the yellow wooly bear, *Spilosoma virginica*. Despite its common name, this caterpillar may be dirty white, light to dark brown, or brownish yellow and may reach 50 mm in length. It feeds on a wide variety of both aquatic and terrestrial plants.

Aphids, *Rhopalosiphum nymphaeae*, may occur in high numbers on waterlettuce. Both adult and nymphs suck plant sap and are found on many species of aquatic plants worldwide (Haag, Habeck, and Buckingham 1985).

Draeculacephala inscripta, a green leafhopper with a pointed yellow head, is common on waterlettuce and other aquatic plants (Haag, Habeck, and Buckingham 1985). This leafhopper, as well as the aphids, may be of importance in transmitting viruses from plant to plant.

Several weevil species were collected from waterlettuce, including the waterhyacinth weevils and the duckweed weevil, but no *Neohydronomus pulchellus* weevils were found.

The first shipment of *N. pulchellus* from Australia arrived in late September 1985. Considerable information was already available on biology and host specificity because of the work in Argentina by DeLoach, DeLoach, and Cordo

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(1976). *Neohydronomus pulchellus* is a small weevil. Males average slightly less than 2 mm long, and females slightly more. They vary considerably in color, ranging from brown to bluish-gray. Adults feed on the leaves, making characteristic round holes. Near the apex, where the leaf is thinnest, these holes completely penetrate the leaves. On the thicker parts of the leaves, the holes penetrate only one surface. Eggs are generally laid in the outer third of the leaves where the female punctures the surface, lays an egg in the puncture, and covers it with a dark substance. Larvae hatch, begin mining, and have three instars. They complete their larval development and pupate within the leaves. Development from oviposition to adult emergence requires about a month.

The weevil was tested for host specificity in quarantine using nonreplicated no-choice tests. Thirty-seven plant species representing 24 families were tested individually in plastic petri dishes 150 mm in diameter and 22 mm deep. The bottom was covered with a water-saturated, size 14, Hercules clarifying filter disc. Whole or partial plants were placed in the dish, usually with the stem and inserted into a hole in the filter disc. The duckweeds, azolla, and salvinia were tested similarly but in small petri dishes 33 mm wide and 10 mm deep. Each dish contained 5 to 7 mm of water. Plants were checked before use to eliminate extraneous insects and damaged plants. Ten unsexed adults were placed in each dish, and the dishes were kept in the quarantine greenhouse where the weevil colony was being maintained. Each test continued for 10 days. Plants were checked every 3 to 5 days for signs of feeding and oviposition. Dead weevils were replaced with live ones, as were plants that deteriorated. Replaced plants and all plants at the end of 10 days were examined under a microscope for signs of feeding and oviposition.

No attempt was made to quantify feeding damage since the plants were so diverse. In most cases, weevils seemed disinterested in the plants and were found around the edges of the dishes. Sometimes they hid under the leaves even when they did not feed.

Feeding and oviposition were always observed on waterlettuce (Table 1). The weevils fed on the duckweeds *Lemna minor* and *Spirodela punctata*; frogbit *Limnobium spongia*; golden club, *Orontium aquaticum*; azolla, *Azolla caroliniana*; and salvinia, *Salvinia rotundifolia*. Feeding on the last two species was very slight. Feeding on goldenclub was confined to the cut end of the petiole where a weevil had made a small hole about 2 mm deep. The leaf was replaced with a new leaf, and no further feeding occurred. All the feeding on frogbit occurred in the spongy tissue on the underside of the leaf. These plants were not in water; thus the underside of the leaves were inaccessible. They probably would not have fed on the leaves in their normal position on the water surface since the weevils apparently do not enter the water. Feeding on duckweed was characterized by the presence of small holes in the dorsal surface of the duckweed leaves.

Eggs were observed on five plant species including waterlettuce. An egg was found on the leaf of panda plant, *Kalanchoe tomentosa*, although no feeding was observed. One egg was found on frogbit, one on azolla, and four on salvinia. None of these eggs were placed in a puncture or otherwise deposited in a normal way.

Table 1
Plants Included in Host-Specificy Test for Adult *Neohydronomus pulchellus* Hustache in Argentina and Florida

Plants Tested			Location*	
Family	Genus and Species	Common Name	Argentina	Florida
Alismataceae	*Sagittarria montevidensis* Cham & Schlect.	Arrowhead	--	--
Amaranthaceae	*Alternanthera philoxeroides* (Mart.) Griseb.	Alligatorweed	--	--
Amaryllidaceae	*Agapanthus africanus* (L.) Hoffmgg.	African lily	--	
Anacardiaceae	*Mangifera indica* L.	Mango		--
Araceae	*Aglaonema* sp.	Aglaonema		--
	Arisaema dracontium (L.) Schott	Green dragon		--
	Dieffenbachia sp.	Dumb cane		--
	Orontium aquaticum L.	Goldenclub		--
	Peltandra virginica (L.) Schott & Endl.	Green arum		--
	Pistia stratiotes L.	Waterlettuce	+++	+++
	Spathiphyllum sp.	Spathe flower		--
Balsaminaceae	*Impatiens balsamina* L.	Impatiens		--
Bromeliaceae	*Ananas comosus* (L.) Merr	Pineapple	--	
Cannaceae	*Canna flaccida* Salisb.	Golden canna		--
Commelinaceae	*Commelina coelestis* Willd.	Dayflower	+	
	Commelina virginica L.	Dayflower	+	
Compositae	*Bidens mitis* (Michx.) Sherff.			--
	Lactuca sativa L.	Lettuce	+	
	Tradescantia crassifolia Cav.	Spiderwort	--	
	Zebrina pendula Schizl.	Wandering jew	+	
Convulvulaceae	*Ipomoea batatas* (L.) Lam.	Sweetpotato		--
Crassulaceae	*Crassula argenta* Thunb.	Jade		--
	Kalanchoe tomentosa Baker	Panda plant		--
Cruciferae	*Brassica oleracea* var. *capitata* L.	Cabbage	--	
	Nasturtium officinale R. Br.	Watercress	--	
Cyperaceae	*Scirpus californicus* (C.A. Mey) Steud.	Bulrush	--	
Graminae	*Oryza sativae* L.	Rice	--	--
	Saccarum officinarum L.	Sugarcane	--	
Haloragaceae	*Myriophyllum aquaticum* (Vell.) Verdc.	Parrotfeather		--
Hydrocharitaceae	*Limnobium spongia* (Bosc.) Steud.	Frogbit		++
	Limnobium stoloniferum (G. W. Meyer) Griseb.	Frogbit	++	
Lemnaceae	*Lemna minor* L.	Duckweed		++
	Spirodela intermedia Koch	Giant duckweed	++	
	Spirodela punctata (Meyer) Thomps.	Duckweed		++
	Lemna sp.	Duckweed	++	
Onograceae	*Ludwigia repens* Forst.			--
	Ludwigia uruguayensis (Camb.) Hara			--
Polygonaceae	*Polygonum densiflorum* Meisn	Smartweed		--
	Rumex sp.	Dock		--
Ponterderiaceae	*Eichhornia azurea* (Swartz) Kunth.	Anchored waterhyacinth	--	
	Eichhornia crassipes (Mart.) Solms	Waterhyacinth	+	
	Pontederia cordata L.	Pickerelweed		--
	Pontederia lanceolata Nutt.	Pickerelweed	+	
	Reussia rotundifolia (L.f.) Castellanos		+	
Potamogetonaceae	*Potamogeton nodosus* Poir.	Pondweed		--
Rosaceae	*Fragaria chiloensis* Duchesne var. *ananassa* Bailey	Strawberry		--

* Symbol definitions are as follows: -- (no feeding); + (very slight feeding); ++ (moderate feeding); and +++ (heavy feeding).

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Table 1 (Concluded)

Family	Genus and Species	Common Name	Argentina	Florida
	Plants Tested		Location*	
Rutaceae	Citrus paradisi Macfed.	Duncan grapefruit		--
Salviniaceae	Azolla caroliniana Willd.	Watervelvet		--
	Salvinia rotundifloria Willd.	Salvinia		--
Saururaceae	Saururus cernuus L.	Lizard's tail		--
Solanaceae	Lycopersicon esculentum Mill.	Tomato		--
Typhaceae	Typha latifolia L.	Cattail	--	
	Typha domingensis Pers.	Southern cattail		--
Umbelliferae	Cicuta mexicana Coult & Rose	Water hemlock		--
	Hydrocotyle ranunculoides L.	Water pennywort	--	--
	Hydrocotyle umbellata L.	Water pennywort		--

It appeared that the female weevil had just dropped the eggs. The eggs on duckweed were placed in punctures on the dorsal surface of the leaf. However, because the leaves were so small, less than one-third of an egg could be inserted. Larvae would be unable to complete their development in leaves as small as duckweed. For that reason *Wolfia* and *Wolfiella* were not tested, although it was recommended that they be included in the tests.

All of the aquatic plants on which feeding or oviposition had occurred in the initial tests (Table 1), except *Kalanchoe tomentosa* and *Orontium aquaticum*, were retested in choice tests (Table 2). Whole plants (except for frogbit where two leaves were used) were placed in petri dishes and arranged at random in a plastic shoebox. Twenty-five weevils were placed in each box, and three replicates were run. The boxes were held in an incubator at 80° F and at 16 light:8 dark photoperid. Boxes were checked every few days and plants were replaced as needed. After 10 days the experiment was terminated, and all plants were examined under the microscope for signs of feeding and oviposition. Any plant material removed before the end of the experiment was checked the same way. No feeding or oviposition was observed on any plants except waterlettuce.

Personal communications from Australia (Don Sands, CSIRO, Brisbane) and South Africa (C. J. Cilliers, Plant Protection Research Institute, Pretoria) report that *N. pulchellus* has not been found or observed in the field on any plants except waterlettuce.

The few plants on which the weevils fed are also considered to be undesirable by most aquatic plant managers. When offered a choice between these plants and waterlettuce, the weevils always fed on the waterlettuce. In view of the host specificity exhibited in tests in Argentina, Australia, and Florida, we feel that the weevil is safe to introduce into Florida.

Permission for field release of the weevils was obtained on 14 November 1986. Since population numbers are low, the weevils will be retained in a nonquarantine greenhouse to increase numbers prior to field release. Barring unforeseen problems, initial field releases are projected for spring 1987.

A second insect, *Namangana pectinicornis* (Noctuidae), was received in late September from Thailand. This moth, which also has been placed in the genus

Table 2

Plants Tested in Quarantine in Florida on Which Feeding or Oviposition by *Neohydronomus pulchellus*
Adults Occurred in a No-Choice Test and Subsequently in a Choice Test

Family	Genus and Species	No-Choice Test		Choice Test	
		Feeding	Oviposition*	Feeding	Oviposition*
Acraceae	*Pistia stratiotes*	+	+	+	+
	*Orontium aquaticum***	+	-		
Crassulaceae	*Kalanchoe tomentosa*	-	+		
Hydrocharitaceae	*Limnobium spongia*	+	+	-	-
Lemnaceae	*Lemna minor*	+	+	-	-
	Spirodela punctata	+	+	-	-
Salvinaceae	*Azolla carolinianat*†	+	+	-	-
	Salvinia rotundifolia†	+	+	-	-

NOTE: Symbols "+" and "-" indicate that feeding or oviposition was observed or not observed, respectively.

　　* Oviposition appeared accidental or atypical on all plants except *Pistia*.

　　** Feeding was observed 2 to 3 mm deep in broken petiole end.

　　† Slight feeding was observed.

Episammia, probably belongs in the genus *Athetis*. Research in Thailand indicates that this insect is host specific to waterlettuce (Suasa-ard and Napompeth 1976). The caterpillars are about 25 mm long when full grown and pupate in the leaves. Initial observations are that this caterpillar is highly destructive to waterlettuce and host-specificity testing in quarantine is expected to begin soon.

ACKNOWLEDGMENTS

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An Overview of the Use of Triploid Grass Carp (*Ctenopharyngodon idella*) as a Biological Control of Aquatic Macrophytes in Devils Lake, Oregon*

by
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Scott A. Bonar,* and Karen L. Bowers**

DESCRIPTION OF THE PROBLEM

The controversy surrounding this project is similar to that outlined by Pauley et al. (1985b), in Oregon has banned the use of the white amur or grass carp (*Ctenopharyngodon idella*) as a potentially risky management tool. However, the grass carp offers a very attractive alternative aquatic weed control mechanism compared to mechanical removal and chemical elimination of the plants.

Devils Lake is a large lake (680 acres) with a watershed of about 12 square miles. The lake is heavily infested with a variety of aquatic plants because the lake is shallow (mean depth 10 ft; maximum depth 21 ft). The ultimate goal of this work is to understand how to control, not eliminate, aquatic macrophytes in the lake. To accomplish this, the Washington Cooperative Fishery Research Unit (WCFRU) has been working to determine a stocking rate that will reduce the aquatic plants to a level acceptable to the Devils Lake Water Improvement District (DLWID) and at the same time ensure that other aspects of the ecosystem are kept in balance. However, potential problems associated with grass carp introductions exist as the consumption of aquatic macrophytes by grass carp can affect the ecosystem in many ways.

The amount and composition of grass carp fecal material will have a direct impact on concentrations of nutrients in the water column, which in turn determines the response of the lake ecosystem. After processing large quantities of macrophytes into grass carp feces, water chemistry has the potential to change and cause blue-green algal blooms (Opuszynski 1979). However, other studies have not demonstrated significant differences in total phytoplankton numbers between test ponds with grass carp and control ponds without grass carp (Lembi et al. 1978). Therefore, the availability of released plant nutrients to other components of the ecosystem needs to be monitored and the effects documented at least for individual geographic areas.

Grass carp also may alter the existing food web. Alterations to macrophyte communities may indirectly affect zooplankton and fish populations. Terrell (1982) postulated that removal of macrophytes by grass carp should result in stimulation of plankton communities. What she found were significantly lower numbers of phytoplankton and zooplankton organisms and genera in ponds containing grass carp versus control ponds.

Populations of prey fish species became more vulnerable to predation, and game fish species have been shown to increase after introduction of grass carp (Bauer, Buck, and Rose 1979). However, Bailey (1978) reports that grass carp control of macrophytes did not affect centrarchid game fish populations or condition factors.

A triploid strain of grass carp (TGC) has been developed recently that is not significantly different morphologically from the diploid fish (Bonar et al. 1985), but is not capable of reproducing, and therefore will not overpopulate a water system. Using computer simulation models, an introduction of a known number of triploid grass carp will yield a predictable macrophyte consumption rate (Wiley et al. 1984a, 1984b). Ideally, macrophytes should be controlled at a level that will minimize harmful effects on other organisms (especially fish and waterfowl) and yield maximum sport fish production (Figure 1).

Recent studies have shown that predatory success for largemouth bass declines as habitat complexity increases, with the mitigating effect on the preferred prey species on the bass, possibly resulting in changes in the overall composition of the prey community (Savino and Stein 1982; Anderson 1984; Wiley et al. 1984a). Hence, introducing a predetermined density of TGC should control macrophytes at the desired percent littoral zone coverage. The percent of macrophyte coverage can be manipulated by varying the density of TGC introduced. However, the effects
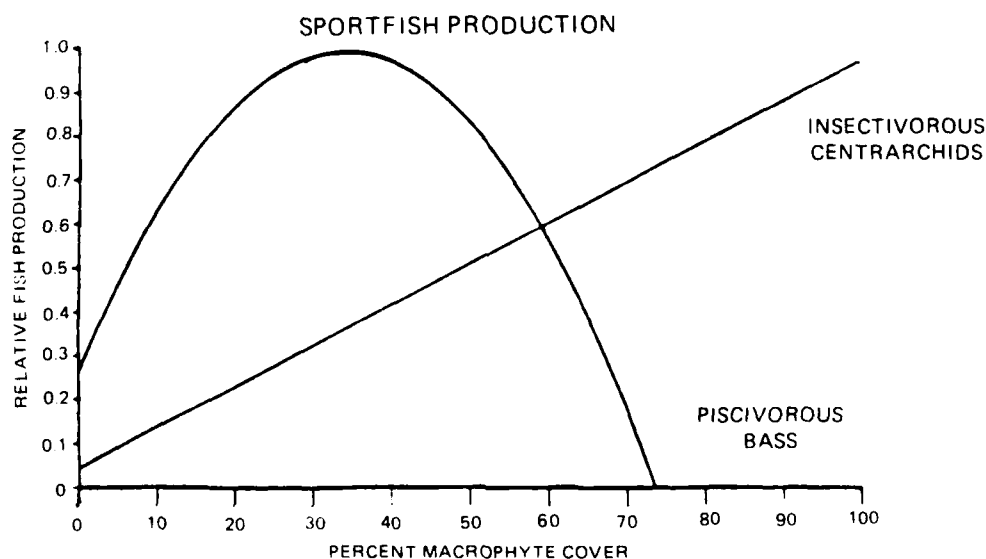

Figure 1. Relative production of piscivorous largemouth bass and insectivorous centrarchids as a function of macrophyte cover. Optimal macrophyte cover for bass production is 30 to 40 percent. (Modified from a trophic dynamic model and field data by Wiley et al. 1984b)

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of the TGC on the biota of systems into which it has been introduced are not well known, and model use in the Pacific Northwest is in its infancy. Data from diploid grass carp studies are often used. As a result, extrapolation of information from diploid grass carp data decreases the ability to accurately predict stocking outcomes. Therefore, as much data as possible must be gathered by studying triploid grass carp to increase the accuracy of the prediction.

PROJECT DESCRIPTION

Methods used will be similar to those outlined by Pauley et al. (1985a). This study will qualitatively and quantitatively evaluate various aspects of: (a) the macrophytes in the lake, (b) the resident game fish, and (c) the water quality and phytoplankton. Additionally, WCFRU will coordinate with the Oregon Department of Environmental Quality (DEQ), the Oregon Department of Fish and Wildlife (ODFW), and the US Fish and Wildlife Service (USFWS) to evaluate the possible effects of TGC introduction into Devils Lake on other fish, wildlife and waterfowl, and their habitats. Some background data on various aspects of Devils Lake are available (Liao and Grant 1983).

Macrophyte monitoring

A BACI (Before-After-Control-Treatment) experimental design will be used to monitor the effects of triploid grass carp on the aquatic macrophytes. The use of the BACI design necessitates the implementation of one or more exclosures to form control areas inaccessible to feeding grass carp. A small mesh net will be used to construct these exclosures. Location, number, and size of the exclosures will be dependent upon evaluation of the first year of baseline data, as aquatic habitat and organisms inside the exclosures must be representative of the entire lake. The lake proper with the introduced grass carp will serve as a treatment group.

An accepted method of monitoring macrophytes is to measure biomass per unit area with a quadrant sampling scheme, using scuba gear and an epibenthic sampler (Purkerson and David 1975). Collected samples are then sorted to species, and biomass per unit area is estimated using wet and dry weights of the macrophyte samples (Pauley et al. 1985a).

We propose to use a modification of this method in the following manner during the baseline year. First, macrophyte density will be estimated by examining aerial photographs taken by USFWS, DEQ, and DLWID for plant density (number per square metre) with a microdensitometer spectrophotometer (Gustafson and Adams 1973, Leonard 1984). Second, macrophyte density and distrubution will be estimated with hydroacoustic techniques (Thomas et al. 1985). A calibrated sonar system designed for the quantitative measurement of fish biomass or density will be used, since the sound echo returned from a target (fish or plant) is proportional to its biomass (Thorne 1984). The survey design will utilize orthogonally spaced line transects. Data will be processed subsequently with an echo integrator to quantitatively measure plant density (Thorne 1984). Both of these methods are experimental and have to be field tested and compared with accepted density

measurement methods obtained from scuba and epibenthic sampling. This field testing will verify that estimates made by these "remote" methods actually represent the macrophyte density (i.e., this will ground truth the aerial photograph and hydroacoustic techniques to allow approximation of density relationships).

After field testing during the baseline year, the experimental method with the least error will be used, if appropriate, with a minimal amount of scuba and epibenthic ground truth verification during the following evaluation years. Using this method, monitoring of macrophyte removal should be possible at any desired time interval and at a greatly reduced cost compared to intensive scuba and epibenthic sampling. A statistically sound comparison will be made of macrophyte biomass in the control and experimental groups. For the macrophyte surveys conducted in 1986, mean biomass per square metre was highest at 181.6 g/m^2 in mid-July. The plant community is dominated by Brazilian water weed (*Elodea densa*), coontail (*Ceratophyllum demersum*), and Eurasian watermilfoil (*Myriophyllum spicatum*).

Stocking rate estimates

Three computer models were examined, tested, and compared for applicability to Devils Lake. Of these, only the Illinois Natural History Survey Model (Wiley et al. 1984b) was determined appropriate for use on Devils Lake. After evaluation of estimates produced by the model, it was concluded that it would be inappropriate to base the Devils Lake stocking rates on the model exactly as it was designed. All parties (DLWID, DEQ, ODFW, EPA, and USFWS) have agreed a stocking rate that does not exceed a number equal to the lowest value obtained by WCFRU calculations plus 15 percent, because of the uncertainty and variance in the estimation procedure. For a detailed discussion of the stocking rate method used for Devils Lake, see Bonar, Thomas, and Pauley (1987).

Feeding rate experiments

Feeding preferences and consumption rates on the aquatic plants found in Devils Lake are necessary for estimating the grazing impact of the grass carp. Preliminary laboratory experiments indicate that grass carp preference for major plants in Devils Lake is as follows, with the most preferred plants listed first: (a) pondweed (*Potomageton zosteriformes*), (b) waterweed (*Elodea canadensis*), (c) water celery (*Vallisneria* sp.), (d) Eurasian watermilfoil (*Myriophyllum spicatum*), (e) coontail (*Ceratophyllum demersum*), and (f) Brazilian waterweed (*Elodea densa*). These tests were conducted in the manner discussed by Bowers, Pauley, and Thomas (1987).

Water quality

The following parameters will be monitored: total and soluble reactive phosphate, nitrate, nitrite, ammonia, organic nitrogen, pH, temperature, dissolved oxygen, chlorophyll *a*, and representative alkalinity. In addition, secchi disk transparancy and suspended solids will be monitored. Algae will be identified to the genus level, and biomass will be reported by genus.

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Resident fish populations

Fish will be collected with plankton nets, minnow traps, beach seines, trammel nets, gill nets, electroshocking gear, hook-and-line, and various combinations of the above gear depending on location and concentration of the macrophytes. Extreme caution will be used to capture fish and release them unharmed. Length frequency distributions and weight-length correlations will be determined by taking length and weight measurements from all fish captured (Ricker 1975). Isometric and/or allometric condition factors will be calculated using weight and length measurements (Ricker 1975). Age distributions and growth characteristics will be estimated using scale analysis and back calculation techniques, respectively (Bagenal 1978). Spatial and temporal distributions will be determined from results of capture methods. Relative abundance will be made for possible game fish species in the lake based on catch per unit effort (Gonyea 1979). Movement and feeding habits will be determined as previously (Pflug and Pauley 1983, 1984).

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Estimation of Triploid White Amur Stocking Densities for Aquatic Plant Control for Devils Lake, Oregon*

by

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INTRODUCTION

Grass carp (*Ctenopharyngodon idella*) have been used as a management tool to control a wide variety of nuisance aquatic macrophytes, primarily in the South, since they were introduced to the United States in the early 1960's (Guillory and Gasaway 1978). The treatment of aquatic plant problems with grass carp has met with mixed results, while some studies have shown the fish to be a biological hazard (Ware and Gasaway 1976, Gasaway and Drda 1977, Forester and Avault 1978) and others consider it an economical, effective alternative to some of the more conventional forms of macrophyte control such as herbicides or mechanical harvesting (Mitzner 1977, Miller and Decell 1984, Wiley and Gorden 1984).

Although such controversy limits generalizations that can be made about grass carp use for aquatic plant control, one assumption commonly held is that the control of aquatic plants at some reduced percentage of their nuisance level, in lieu of the eradication of all plants in a water body, minimizes the harmful environmental effects and maximizes the management benefits. The goal of this research is to determine the crass carp stocking rates for control of aquatic plants in the Pacific Northwest waters.

In the early 1970's Northwest management agencies discovered that nuisance growths of Eurasian watermilfoil (*Myriophyllum spicatum*) and other aquatic plants caused environmental and economic damage to inland waters. Unfortunately, the traditional plant control method of using herbicides was becoming less acceptable to the public, and mechanical removal was considered inefficient and costly. In 1983, studies were initiated to assess the cost effectiveness and the environmental impact of the use of grass carp for aquatic plant control in the State of Washington. These studies are being conducted in seven northwest lakes and consist of 1 to 2 years of baseline data collection followed by several years of lake monitoring after the introduction of the carp. In 1986, Oregon introduced the first grass carp into an inland water body for aquatic plant control purposes and initiated a monitoring program to evaluate their impact (Pauley et al. 1987).

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Determining a reasonable amount of grass carp to stock to achieve the desired amount of plant control has proved to be a complex problem. The Pacific Northwest differs from other areas in the United States in which the grass carp has been used previously. Northwest temperature regimes and plant community compositions approximate those of northern Europe, and the most problematic plant infestations consist of macrophytes that several researchers have found to be less palatable to the grass carp, such as Eurasian watermilfoil (Leslie et al., in preparation; Fowler and Robson 1978). Although the use of low stocking rates which have been successful in southern lakes is tempting to managers in the Pacific Northwest, an analysis of the feeding and growth of the grass carp in the cooler waters and on the different plant species available in the Northwest suggests that the low southern stocking rates may be inadequate for plant control in these waters.

In 1986 the task of determining white amur stocking rates for Devils Lake, Oregon, was initiated. This lake represents the first official stocking of a Pacific Northwest lake with triploid grass carp. Since little data exist describing the behavior of this fish in the Pacific Northwest, information from the literature (case histories) and simulations with grazing models that were developed outside the Pacific Northwest were used to estimate the amount of fish needed to control the plant infestation in Devils Lake. This paper discusses the methods of predicting stocking rates in the Pacific Northwest lakes and in Devils Lake.

Study area description

Devils Lake is a shallow, polymictic, marine climate lake on the central Oregon coast. It has a surface area of 275 ha, a maximum depth of 6.4 m, and a mean depth of 3.0 m. The lake is fed by two small streams, Rock Creek and Thompson Creek, and has an outlet to the Pacific Ocean, the D River, which is claimed as the shortest river in the world. The mean seasonal water temperature of Devils Lake averaged over 4 years (1982-1986) was 19.1° C.

In 1986, the aquatic plant community covered about 55 percent of the lake's total surface area and consisted primarily of four submersed plant species: 16 percent *M. spicatum*, 23 percent *Ceratophyllum demersum*, 6 percent *Elodea canadensis*, and 34 percent *Elodea densa* (Figure 1). The biomass of the aquatic plant community had a maximum seasonal fresh weight of 1,652 g/m^2 (Figure 2). The plant biomass in the lake does not completely die off in the winter, probably because of the buffering effect of the marine climate influence on the winter weather patterns.

Devils Lake is in an exclusive recreational/retirement area and supports significant tourism. The shoreline is relatively well developed, with residences and some resorts. Excessive nutrient loading from septic tanks, dairy farms, and runoff from developed areas qualify this lake as eutrophic. Water sports on the lake, led by sportsfishing, are very important to tourism and local property values. In the past few years the lake has been avoided by sportsfishing clubs after Memorial Day because of the enormous seasonal increase in aquatic plants. Other aquatic sports such as swimming and boating have suffered dramatically, causing an estimated seasonal loss of $1 million. In 1986 the Devils Lake Water Improvement District stocked 10,000 triploid grass carp (ca. 13 kg/vegetated ha). A subsequent
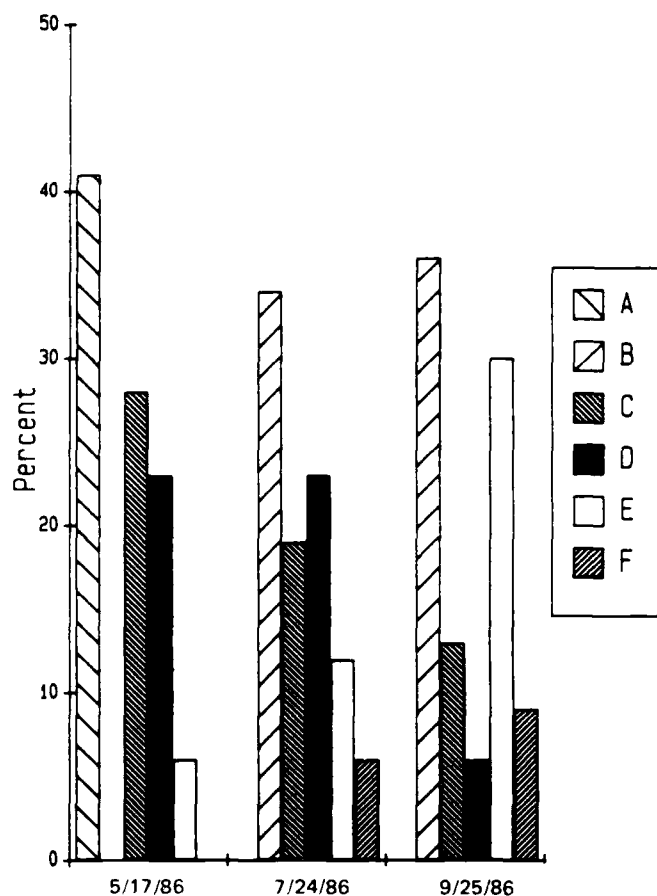
Figure 1. Percent composition of aquatic macrophytes in Devils Lake, Oregon. Macrophytes are as follows: *Elodea* spp. (A), *Elodea densa* (B), *Myriophyllum spicatum* (C), *Ceratophyllum demersum* (D), miscellaneous algae and decayed plant material (E), and *Elodea canadensis (F)*

Figure 2. Mean wet weight (spun) of aquatic macrophytes with 95-percent confidence intervals in Devils Lake, Oregon. Symbol interpretation is as follows: *Elodea* spp. (●), *Elodea densa* (▼), *Elodea canadensis* (■), *Myriophyllum spicatum* (◆), *Ceratophyllum demersum* (◆), Miscellaneous algae and decayed plant material (▼), and total plant biomass (■)

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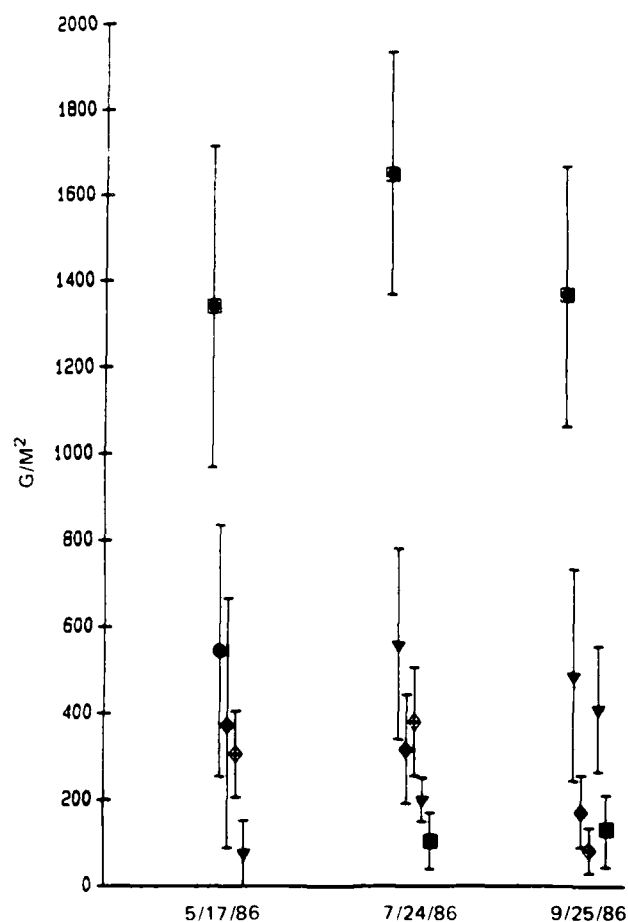
stocking will take place in the spring of 1987 in accordance with the recommendations set forth in this paper.

The primary environmental concerns associated with the release of grass carp are: (a) the lake is eutrophic and there is the possibility of aggravating this condition by the release of nutrients now contained in the aquatic plant community, via grass carp grazing and (b) the lake is a primary overwintering area for waterfowl that are believed to be partially dependent upon the aquatic macrophytes and their associated invertebrates for food.

DETERMINATION OF STOCKING RATES FOR DEVILS LAKE

Stocking Devils Lake for control rather than eradication of aquatic plants was chosen to minimize the potential for catastrophic environmental impacts. Unfortunately, understocking in mixed-plant assemblages may cause a shift to unpalatable plant species with no net control of the lake's total biomass (Fowler and Robson 1978). In contrast, overstocking may cause the eradication of all the plants in the lake and possible negative environmental effects. Of the two methods, understocking is a more preferable management alternative because it has the simple solution of the addition of more fish. Overstocking requires the removal of the excess fish at a high degree of expense and difficulty.

Examples of the variability of control achieved with different stocking rates in different areas (Table 1 and Figure 3), reflect the management goals and local environment conditions of widely separated geographic regions. The left side of Figure 3 shows low stocking rates generally required for plant control in waters in southern regions. Researchers in Florida have found as few as 1 to 2 fish/metric ton of vegetation used in combination with herbicides and mechanical harvesting suitable for controlling stands of *Hydrilla* sp. (Leslie et al., in preparation). In general, these low subtropical rates have not been used for control of northern aquatic plant communities. The right side of Figure 3 illustrates the higher stocking rates that have been used for plant control in the temperate climates of Northern Europe.

Lake managers can estimate a reasonable number of grass carp to stock examining successful stocking rates from previous studies or by using one of several available stocking rate models. To arrive at a stocking rate for Devils Lake, we originally used a model developed by the Illinois Natural History Survey (INHS) for determining stocking rates in the State of Illinois (Wiley and Gorden 1985). This model was developed using data from both extensive laboratory experiments and many 1-year field experiments. Although short-term field experiments were used to develop and test this model, it has not been refined with long-term information from the field.

Stocking rates recommended by the INHS model for three common aquatic macrophytes found in the Pacific Northwest demonstrate variability similar to that of successful stocking rates used in different regions of the world (Table 1). The rates calculated by the model for controlling *Potamogeton pectinatus, Elodea canadensis*, and *Myriophyllum spicatum* at a level of 20 to 40 percent of the original

Table 1
Stocking Rates for Various Locations

Case	Location	Stocking Rate*	Fish Size g	Plants Present	Results	Study Length years	Reference
1	Ramona L., Co!orado	11*	34-2,244	7	C	2	Swanson 1986
2	Lake Bell, Florida	22	227	1	E	5	Van Dyke, Leslie, and Nall 1984
3	Pond 6 Missouri	29*	370	2, 3	N	1/3	Rottman and Anderson 1976
4	Turkemania USSR	31	148	4	E	1/3	Aliev 1963
5	Pond 7, Missouri	35*	507	2, 3	E	1/3	Rottman and Anderson 1976
6	Waihi Beach Reservoir, New Zealand	55*	2,102	5, 6	S	2	Mitchell 1980
7	Netherlands (average recommended rate, 1986)	57*	400-600	7, 20	C	--	R. E. Riemens, pers. comm.
8	Devils Lake, Lincoln City, Oregon	60	200	4, 7, 8, 9	C	--	--
9	Dgal Wielki, Northern Poland	62	142-6,046	3, 4, 7, 9	E	12	Krzywosz and Radziej 1980
10	Pond A-4, Ferdinand, Indiana	69	481	2, 10, 11,12	E	1/3	Lembi et al. 1978
11	Red Haw Lake, Iowa	90	380	2, 14	C	3-1/2	Mitzner 1977
12	Netherlands (average rate, privately owned lakes, 1977-81)	107*	300-500	--	C	Average of rates used for 5 years	Riemens 1982
13	Pond 2 England	116*	200 400	4, 14, 19	S	1-1/2	Fowler and Robson 1978
14	Waihi Beach reservoir, New Zealand	122*	2,216	6	E	1-1/2	Mitchell 1980
15	Netherlands (average rate sportfishing waters, 1977-81)	139*	300-500	--	C	Average rates used for 5 years	Riemens 1982
16	Netherlands (average rate, municipal waters, 1977-81)	150*	300-500	--	C	Average of rates used for 5 years	Riemens 1982

* Values noted with asterisk are expressed in kilograms per hectare, since rates in kilograms per vegetated acre could not be obtained for all studies.

** Plants are coded as follows: 1. *Hydrilla* sp., 2. *Najas* sp., 3. *Chara* sp., 4. *Myriophyllum spicatum*, 5. *Potamogeton ochreatus*, 6. *Nitella hookeri*, 7. *Elodea canadensis*, 8. *Elodea densa*, 9. *Ceratophyllum demersum*, 10. *Myriophyllum verticillatum*, 11. *Potamogeton crispus*, 12. *Potamogeton foliosus*, 13. *Elodea ernstae*, 14. *Potamogeton pectinatus*, 15. *Callitriche stagnalis*, 16. *Nasturtium officinale*, 17. *Polygonum decipiens*, 18. *Eleocharis sphacelata*, 19. *Potamogeton natans*, and 20. filamentous algae.

† Result codes are as follows: C = control to 10 to 50 percent biomass of the original, E = eradication, N = no significant effect, S = shift to non-preferred plant species.

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Table 1 (Concluded)

Case	Location	Stocking Rate*	Fish Size g	Plants Present	Results	Study Length years	Reference
17	Parkinson's Lake, New Zealand	150*	--	8, 18	E	3-1/2	Mitchell 1980
18	England (average recommended rate)	160*	--	--	C	--	Stott and Buckley 1978
19	East Germany (average recommended rate)	200*	250-400	--	E	1	Jahnichen 1973
20	Netherlands (average rate used by waterboards, 1977-81)	223*	300-500	--	E	Average of rates used for 5 years	Riemens 1982
21	Chichester Canal, England	324*	300-500	13	N	4	Mugridge et al. 1982
22	Tsyrupinsk Farm, USSR	450*	--	--	E	1/3	Lupacheva 1968
23	Pond 4 England	479*	--	4, 14, 19	S	1-1/2	Fowler and Robson 1978
24	Ditch, Bay of Plenty, New Zealand	500*	980	15, 16, 17	S	1/2	Edwards and Moore 1975
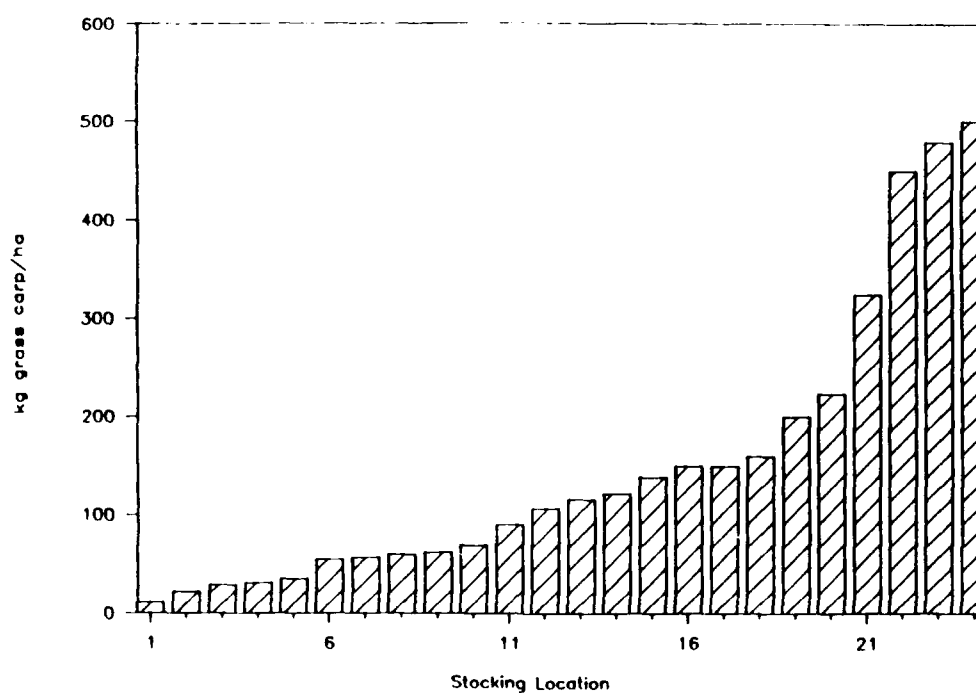
Figure 3. Stocking rates from several grass carp treatments. Stocking location numbers correspond to case numbers in Table 1.

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within 4 years were 20, 18, and 130 kg/vegetated ha, respectively. The high variation in these stocking rate predictions was due primarily to large differences in grass carp consumption rates for the different plant species.

The flexibility of the INHS model allowed most of the variables describing the Devils Lake plant community dynamics and temperature regime to be used in the calculation of a stocking rate. Plant biomass data were collected from Devils Lake one season before stocking and input into the model. Water temperatures for 4 years prestocking were available, so these were averaged to give a temperature regime that could drive the model. The temperature at which plant growth was initiated, the amount of overwintering, plant material, and the mortality of the grass carp were estimated by using a combination of limited observational data from Devils Lake and default data from Illinois.

Grass carp consumption rates of aquatic macrophytes entered into the model were predicted for Devils Lake by calculating a weighted average of the consumption rates of all plant species in the lake determined from Illinois, Washington, and Arkansas laboratory studies (Stanley 1974; Wiley and Gorden 1985; Bowers, Pauley, and Thomas 1987). This produced an estimate of a stocking rate for Devils Lake, which was compared with the temperate water lake case histories available in the literature.

RESULTS AND DISCUSSION

Stocking rate estimates made with the INHS model indicated that 56,250 fish weighing 0.2 kg each could control the aquatic macrophytes in Devils Lake within 5 years. These estimates were high compared to the southern United States case histories, but were reasonable when compared to European case histories from more northern temperature lakes. However, analysis of the most recent plant biomass and growth data from Devils Lake and changes made in the model by Illinois researchers, which predict a lower stocking rate, suggest that additional modifications are necessary before using this program for estimating Northwest rates. This conclusion is documented by recent literature and personal communications with European researchers who now support the use of lower stocking rates for plant control. Therefore, until we can run simulations with the updated version of the IHNS model, stocking rates for Devils Lake will have to be based on the most recent case-history analyses from the literature.

Twenty-four case histories of grass carp introductions were analyzed for estimation of a stocking rate. A problem with analyzing the case history data was the lack of standardization of units used to estimate the number of fish stocked per amount of plants. The case histories of grass carp introductions were divided into three categories relative to the statistics that were used to estimate their stocking rates. These statistics, in order of increasing accuracy, were weight of fish stocked (a) per total surface area of the lake, (b) per surface area of vegetation in the lake, and (c) per weight of vegetation in the lake. Unfortunately, the measurement techniques varied, and estimates of precision were not routinely reported. In addition, fish mortality from predation and disease was seldom ascertained and is another source of uncertainty.

Seven case histories for temperate water lakes were found where measurements of plant densities were available to estimate the stocking rate as the ratio of fish-to-plant weight. Three of these case histories resulted in plant control. three in eradication, and one in failure with a shift to unpalatable plants. The average stocking rate for the three lakes that demonstrated plant control was 1.41 kg of grass carp per metric ton of vegetation. Unfortunately, the one plant control failure used a stocking rate of 1.30 kg/metric ton and one eradication of *M. spicatum*, a less preferred plant, occurred at 1.60 kg/metric ton. This provides a very small window to estimate stocking rates on a case-history basis.

Our estimates of plant density in Devils Lake at peak biomass are 16.14 metric ton/ha ± 15 percent. Given the 15-percent confidence limits of the density estimate and the deterministic estimate of 1.41 kg fish/metric ton of vegetation from case-history analysis, we have computed three stocking rate estimates (Table 2). Of these three estimates, we recommended the lowest (27,090 fish) because of the uncertainty we have regarding the estimation procedure. This will result in a spring planting of 17,090 0.2-kg grass carp, since 10,000 fish were planted in Devils Lake in the fall of 1986.

Table 2
Stocking Rate Estimates for Devils Lake

Kilograms Fish/ Metric Ton	Metric Tons Vegetation/Ha. (± 15%)	Kilograms Fish/Ha.	Ha.	Kilograms Fish	Number of 0.2-kg Fish Recommended	Number of Fish To Be Stocked*
1.41	18.56	26.17	280	7,328	36,640	26,640
1.41	16.14	22.76	280	6,372	31,860	21,860
1.41	13.72	19.35	280	5,418	27,090	17,090

* Recommended number minus 10,000.

In view of the uncertainty of the IHNS model predictions, the potential enviromental risks, and the associated high cost of the fish, we feel that use of these recent case histories is the best choice for stocking rate determination at this time. This recommendation does not mean that the use of the INHS simulation model cannot predict accurately; it simply means it has not been developed and field tested sufficiently at this time for use in the Pacific Northwest. The Devils Lake study represents an additional opportunity to determine the effect of grass carp on a large, temperate climate lake. Unfortunately, because of the long-term nature of the grass carp grazing effect (3 to 5 years), the final analysis of this research will not be available until the early 1990's.

The stocking of Devils Lake illustrates some of the challenges of predicting stocking rates for grass carp. Case-history analyses and stocking rate models both demonstrate benefits and drawbacks when used to determine a reasonable amount of grass carp to stock. Although models give predictions that are subject to several sources of error, they allow the user to determine how different variables interact. Stocking rate estimates given by models should be compared with as many actual case histories as possible, since many environmental factors can be crucial when

calculating a stocking rate. Comparisons of the predictions given by the INHS model with case-history data from Europe show that while this model can be used to roughly predict justifiable Northwest rates, it is a long and laborious process to adjust it to local climatic regimes, macrophyte dynamics, and other site-specific characteristics. Similar difficulties exist when trying to use stocking rates predicted from case-history information. Averaging successful stocking rates from several temperate climate lakes probably represents the best way to determine a stocking rate for Devils Lake at this time; however, differences in plant community compositions and water quality could render stocking rates that were effective in the case-history lakes but unsuitable for Devils Lake.

Controlling plants in a multispecies lake with grass carp is difficult because of the tendency of the less preferred macrophytes to recolonize areas from which more preferred species have been removed. Based on many studies of other mixed-species lakes (Fowler and Robson 1978; Leslie et al., in preparation), if the stocking rate in Devils Lake is successful, the carp should sequentially remove the macrophyte species in order of decreasing palatability. The final result would be a monospecific plant community of the least preferred species controlled at some reduced level. Other methods of control, such as mechanical harvesting, may be required on a limited basis to aid the carp in controlling this least palatable species. Use of data and models from outside this area has many limitations. Rapid, accurate predictions for the Pacific Northwest could best be done by using a model designed specifically for Northwest plant assemblages and temperature regimes based on the framework of the INHS model. This is what we will be attempting with our research in Washington State using controlled field introductions to test the accuracy of stocking rate predictions (Pauley et al. 1985).

ACKNOWLEDGMENTS

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Feeding Preference on Pacific Northwest Aquatic Plant Species by Diploid and Triploid Grass Carp (*Ctenopharyngodon idella*)*

by

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INTRODUCTION

This study is part of a program being conducted in Washington State (Pauley et al. 1985b) and in Oregon State (Pauley et al. 1987). The primary objectives of the laboratory feeding experiments conducted in 1985 and 1986 were to: (a) determine the relative macrophyte preference by both diploid and triploid fish, (b) ascertain the influence of water temperatures on macrophyte preference, and (c) examine the preferences of macrophyte species present in six Washington State study ponds and one large lake in Oregon State. Experiments conducted in 1985 with diploid and triploid fish at two different densities showed no difference in relative preference for five macrophyte species based on either ploidy or density (Pauley et al. 1985). The following preference ranking was determined based on the 1985 experiments: (a) watermilfoil (*Myriophyllum spicatum*), (b) coontail (*Ceratophyllum demersum*), (c) bladderwort (*Utricularia vulgaris*), (d) floating-leaf pondweed (*Potamogeton natans*), and (e) watershield (*Brasenia schreberi*). This paper presents the results of the 1986 experiments testing the relative preference of triploid grass carp for 12 macrophyte species from three different Pacific Northwest aquatic macrophyte communities, and the influence of water temperature on preference.

Factors that influence grass carp feeding habits are plant species, water temperature, and fish age (Mickewicz, Sutton, and Blackburn 1972). Though the relationship between water temperature and feeding rate is fairly well studied (Wiley and Gorden 1984), the influence of water temperature upon feeding preference is less well known. The grass carp that will be stocked in the study ponds will be subject to a large range of water temperatures throughout the year (Pauley et al. 1985a). Any alteration of feeding preference due to water temperature could prove critical to the validation of our stocking rate model (Bonar, Thomas, and Pauley 1987). Grass carp are known to feed at water temperatures as low as 10° C (Wiley and Gorden 1984) and can survive temperatures above 33° C (Prowse 1971). It is very likely, then, that geographic

region, as well as local conditions, affects the preference of grass carp for various plants because it is not uncommon for different researchers to observe widely different preferences (Cross 1969; Duthu and Kilgen 1975; Bailey 1978; Fowler and Robson 1978; Young, Monaghan, and Heidinger 1983; Wiley and Gorden 1984; Wiley, Prescitelli, and Wike 1986).

MATERIALS AND METHODS

Temperature-controlled feeding studies were conducted at 15°, 20°, and 25° C on triploid grass carp (TGC). In the 15° C experiments, the fish were held in 400-gal tanks at the Sand Point Naval Facility in Seattle and were maintained by water pumped from a 60-ft depth in Lake Washington, sterilized with ultraviolet light, and sand-filtered prior to use. Two tanks of five triploid fish (mean weight = 265 g, S.D. = 24.7) were used in the experiments, each containing five fish. There was no significant difference in the mean weight of the fish betweeen tanks. Before the experiments began and weekly thereafter, weights of the experimental fish were taken. Growth during the experiments was negligible (<5 g/fish in a 1-month period). Temperature was maintained by altering the flow rate into the tank. The tank temperature was recorded once in the morning and once in the evening. Temperatures ranged between 14^ and 16° C but were maintained at 15° C 80 percent of the time.

For the 20° and 25° C experiments, six fish were kept in a wet lab at the Fisheries Research Institute, Seattle, and were placed, three fish per trough, in 100-gal troughs. There was, however, no significant difference in the mean weight of fish in each trough. Water was heated using standard 100-gal aquarium heaters. Flow rates were reduced to maintain the 25° C temperature, and the thermostat of the room in which the fish were kept was set at 24° C to help reduce cooling from the air. Temperatures higher than 25° C would have required the total cessation of flow into the troughs. Since low oxygen levels are known to have a significant influence on feeding rates and behavior (Prowse 1971), it was felt that results from experiments conducted at temperatures higher than 25° C would be unreliable.

Plants collected from natural ponds were processed for feeding by thorough rinsing to reduce or eliminate periphyton and were then kept alive in a greenhouse situation. Macrophytes planted in gravel-filled pots were used in the circular tanks in two-species preference trials and multiple-species, community preference trials. Macrophytes offered to the fish in the troughs were bundled loosely at their base with every effort being made to ensure a natural appearance, e.g., floating leaved plants were allowed to float. Milfoil (M. spicatum) and coontail (C. demersum) used in these experiments were collected from Lake Washington. Sago pondweed (Potamogeton pectinatus), floating-leaved pondweed (P. natans), watershield (Brasenia schreberi), bladderwort (U. vulgaris), water smartweed (Polygonium amphibium), and curly-leaf pondweed (Potamogeton crispus) were collected from the Washington study ponds where they occur. Devils Lake, Oregon, was the source of Canadian waterweed (Elodea canadensis), Brazilian waterweed (Elodea densa), water celery (Vallisneria americana), and flat-stemmed pondweed (Potamogeton zosteriformes).

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Three macrophyte community assemblages were selected for the community preference trials. East Pipeline Lake, representative of eastern Washignton plant communities, contains large amounts of *M. spicatum*, *P. pectinatus*, *P. crispus*, *P. amphibium*, *C. demersum*, and *E. canadensis*,. The dominant species of Keevies Lake, representative of western Washington plant communities, are *U. vulgaris*, *B. schreberi*, and *P. natans*. Devils Lake, Oregon, represents a large North̄west system with a large number of species but contains predominantly *E. densa*, *E. canadensis*, *M. spicatum*, and *C. demersum*, with *P. zosteriformes* and *V. americana* numerous in several of the lake's shallow bays. The three community assemblages selected—East Pipeline, Keevies, and Devils Lakes—were tested using multiple-species preference trials where fish were offered all macrophyte species from that community at once. Continuous monitoring ensured that no species was entirely consumed and therefore unavailable for selection. Fish were offered all species in that community for a period of 3 days. The Devils Lake species were tested at 15°, 20°, and 25° C. Because all of the species in Keevies Lake and several of the most numerous species (watermilfoil and coontail) in East Pipeline Lake were tested in 1985 at water temperatures around 15° C, those communities were only tested at 20° and 25° C in 1986.

Two-species preference trials were conducted for 24 hr without replacement on the following macrophyte pairs: (a) *U. vulgaris* and *P. zosteriformes*, (b) *P. crispus* and *P. zosteriformes*, (c) *P. crispus* and *P. pectinatus*, (d) *P. amphibium* and *P. natans*, and (e) *B. schreberi* and *E. densa*. Replicate tests were conducted simultaneously in two replicate troughs of three triploid fish each. It was assumed that if species A was preferred to species B, and B was preferred to species C, then A was preferred to C. By this assumption, all 12 species occurring in the three lakes were ranked on the basis of the results from the multiple-species community trials and the 24-hr, two-species trials.

Community preference data were analyzed using a nested ANOVA to test the null hypothesis of no difference in mean consumption due to water temperature (alpha = 0.05 for all statistical tests). The Newman-Keuls multiple range test was used to test the null hypothesis of no difference in mean consumption in grams by macrophyte species and then to compare consumption means to determine which were significantly different. Two-species preference data were analyzed using t-tests. The null hypothesis of no difference between means was tested at the 0.05 level. T-tests were performed using both consumption in grams and percentage consumption data. The percentage data were submitted to the arcsine transformation prior to analysis.

RESULTS

The results of the community feeding studies were clear and, generally, were corroborated by the results from the 1985 and 1986 two-species preference trials, as well as the 1985 multiple-species preference trial, which showed no difference between diploid and triploid fish (Table 1). TGC preference for the plant species in the Keevies Lake plant community was quite apparent, and no further studies are deemed necessary for this community (Table 2). East Pipeline plant species

Table 1
Relative Preference Ranking as a Function of Grams Consumed for Five Pacific Northwest Macrophyte Species Tested in 1985 Using Triploid and Diploid Grass Carp

Species	Diploids, g Total of consumption	Triploids, g Total of consumption
M. spicatum	105.9	111.2
C. demersum	25.3	17.3
U. vulgaris	18.2	17.0
P. natans	0.3	2.5
B. schreberi	0.0	1.6

Note: Fish were offered to all species at once for a period of 3 days.

Table 2
Ranking of Macrophytes from the Keevies Lake Community Feeding Preference Study

Species	Mean Consumption, g	
	20° C	25° C
U. vulgaris	101.6	118.0
P. natans	3.4	1.3
B. schreberi	0.3	0.7

were easily ranked (Table 3), with the exception of *P. pectinatus* and *P. crispus*. Two-species preference trials designed to clarify the TGC's relative preference for these two species proved inconclusive (Table 4), and these two species are considered to have equal electivity. Preference for the Devils Lake plant species demonstrated that *P. zosteriformes* was clearly the most highly preferred species in the lake (Table 5). However, there were insignificant differences between the consumption values obtained for *V. americana* and *E. canadensis* at 25° C and *P. zosteriformes* and *E. canadensis* at 15° C, all highly preferred species. Subsequent two-species preference trials revealed that *E. canadensis* was preferred over *V. americana*. No significant differences in relative preference ranking were found due to water temperatures where the same relative preference for the 12 macrophytes tested in this study occurs at all three water temperatures tested with TGC.

Table 3
Ranking of Macrophytes from the East Pipeline Community Feeding Preference Study

Species	Mean Consumption, g	
	20° C	25° C
P. pectinatus	106.8	122.6
P. crispus	112.5	116.7
E. canadensis	52.7	41.9
M. spicatum	11.5	12.3
C. demersum	8.3	0.0
P. amphibium	4.8	3.8

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Table 4

**Mean Consumption (g) and Mean Percent Consumption of
Macrophytes Tested in Short-Term, Two-Species Preference
Trials**

Pair Tested	Mean Consumption g	Standard Deviation	Percent Consumption
U. vulgaris	20.5	(4.8)	55.3%
P. amphibium	1.5	(2.1)	4.9%
P. crispus	13.0	(1.9)	65.3%
P. zosteriformes	4.5	(1.6)	27.8%
P. crispus	12.9	(3.0)	54.3%
P. pectinatus	12.2	(2.9)	49.4%
P. amphibium*	8.6	(4.5)	43.7%
P. natans	0.0	(0.0)	0.0%
B. schreberi*	4.4	(1.1)	55.2%
E. densa	0.0	(0.0)	0.0%

*Note: No consumption had occurred after 1 hr; therefore, the
experiments were conducted for a period of 6 hr.

Table 5

**Ranking of Macrophytes from the Devils Lake
Community Feeding Preference Study**

Species	Mean Consumption, g		
	15° C	20° C	25° C
P. zosteriformes	63.2	95.1	104.1
E. canadensis	46.9	66.3	37.1
V. americana	16.7	17.6	29.6
M. spicatum	0.0	6.3	0.9
C. demersum	0.0	1.4	0.0
E. densa	0.0	0.0	0.0

The results of the various preference trials were combined to obtain an overall preference ranking for these 12 Northwest macrophyte species (Figure 1). Three groupings were devised for the 12 plant species. Highly preferred plant species were those that were consumed consistently in high quantities by the TGC. Plants in this category were *P. zosteriformes, P. pectinatus, P. crispus, V. americana,* and *E. canadensis.* Moderately or variably preferred species were those that were consumed in quantities that varied, depending on the other plant speicies offered concurrently. Macrophytes falling into this category were *M. spicatum, C. demersum, U. vulgaris,* and *P. amphibium.* Nonpreferred plant species were those that were consistently consumed in very small quantities, indicating that TGC select against these macrophytes. *B. schreberi, P. natans,* and *E. densa* were in this category.

DISCUSSION

In reviewing the literature on grass carp feeding preference, one is struck by the disagreement between authors regarding the preference ranking of different
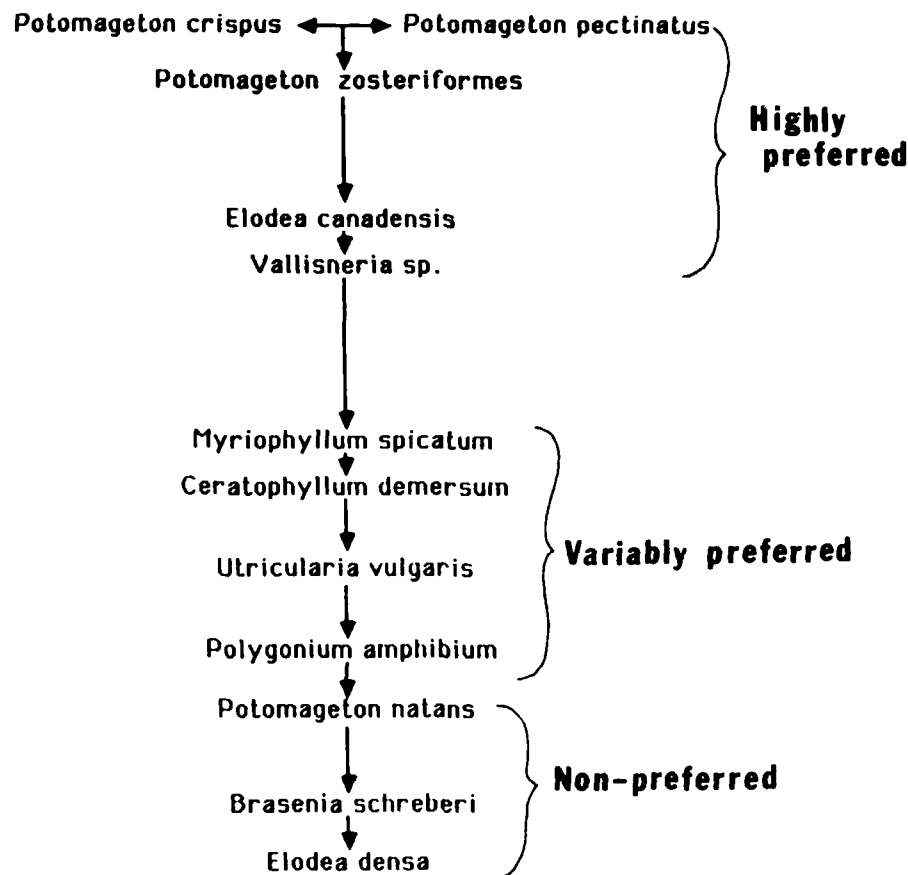
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Figure 1. Overall preference ranking of 12 Pacific Northwest macrophyte species. Triploid grass carp relative preference is shown in descending order

macrophytes. However, the preference hierarchy presented in this report generally is similar to that presented by several other authors. Fowler and Robson (1978) found that diploid grass carp consumed *E. canadensis* and *P. pectinatus* readily, while *M. spicatum* and *P. natans* were listed as nonpreferred. Duthu and Kilgen (1975) ranked *P. pectinatus* over *C. demersum*. Young, Managhan, and Heidinger (1983) reported that hybrid grass carp preferred *E. nuttali* (a similar species to *E. canadensis*) over *C. demersum*. Bailey (1978) found that stocked grass carp quickly removed *C. demersum*, *Utricularia* sp., and several *Potamogeton* spp. from a lake in Arkansas, while leaving *B. schreberi* relatively untouched. These results seem to agree with the data presented in this paper. It should be pointed out that Bailey (1978) also found *E. densa* was eliminated very quickly from the lake as well. Wiley and Gorden (1984) observed a slightly different order among three of our highly preferred species, with *E. canadensis* ranked above *P. pectinatus* and *P. crispus*, although their data supported our conclusion that *M. spicatum* is preferred to *C. demersum*. Wiley and Gorden (1984) stated that grass carp had an extremely high feeding rate upon lettuce, which is a food low in caloric content, suggesting that nutrient value could have a profound effect on feeding rates. A major conflict to our preference ranking data comes from Cross (1969), who ranks *M. spicatum* and *P. natans* as being highly preferred plants.

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Possible explanations for the variance of preferential feeding behavior found in grass carp include abundance and handling time of the plant, chemical and nutrient content of the plant, the ambient habitat of the macrophyte food, and the size of the fish. Experiments designed to test the influence of nutrient content upon preference have revealed no evidence of such a relationship (Wiley and Gorden 1984). Prowse (1971) states that plants with a high selectability are typically low in fiber, while fibrous plants are invariably selected against. Fibrous plants can be controlled through grass carp stocking if the carp are stocked in the spring and feed upon the young shoots or buds before much growth occurs.

Utricularia sp. is known to be a poor growth food for juvenile grass carp (Varghese, Devaraj, and Shantharam 1976). Grass carp fed a monoculture of *C. demersum* exhibited poor growth, skeletal deformity, and a high incidence of disease (Cassani and Caton 1983). Therefore, plants may have been selected against based on their nutrient value.

Searching time and processing time may be functions of both the environment and the size of the fish. Colle, Shireman, and Rottmann (1978) has indicated that size of the grass carp may play a role in its plant preference. Fischer (1968) has indicated that the morphological structure of the plants and the development of the mouth apparatus are responsible for plant selection by young grass carp.

Although no temperature differences were detected in our preference trials, our lowest temperature tested was 15° C. Colle, Shireman, and Rottmann (1978) has indicated that feeding preference may not be influenced until it drops below 14° C, at which time feeding rate is greatly reduced as well.

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White Amur Research in Reservoir Embayments
of the Tennessee River System

by
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In 1983 the Tennessee Valley Authority began a large-scale study using the white amur for aquatic weed control. A 400-acre embayment (Town Creek embayment) on Guntersville Reservoir in northwest Alabama and an 11.5-acre embayment on Melton Hill Reservoir in eastern Tennessee were selected as representative study sites. Baseline data were collected during the 1984 growing season, and poststocking data were collected in 1985 and 1986.

A monosex population of white amur was stocked in Town Creek in September 1984 at a rate of 18 fish/weed-infested acre or 11 fish/surface acre, while sterile triploid amur were stocked in the Melton Hill site at a rate of 24 fish/weed-infested acre or 17 fish/surface acre. Inadvertant removal of the screen barrier and loss of some fish required an additional 165 triploid amur to be stocked at the Melton Hill site in July 1985. The amur at the Town Creek site had an average weight of about 0.6 lb at stocking and weighed approximately 12 lb by August 1986.

Early in 1985, annual species such as spinyleaf naiad, southern naiad, muskgrass, and narrow-leaved pondweeds disappeared from the drawdown zone at the Town Creek site, apparently as a result of a selective feeding preference by the white amur. Scattered colonies of American pondweed, curly-leaf pondweed, and hydrilla also were eliminated, leaving a montypic community consisting of Eurasian watermilfoil. Although more than 250 acres of Eurasian watermilfoil were still present in July 1986, regression analysis showed a significant decrease in standing crop of watermilfoil from 1984 to August 1986. A slight increase in standing crop of watermilfoil occurred in a nearby control embayment on Guntersville Reservoir. At the Melton Hill site, watermilfoil and other submersed macrophytes were eliminated during the 1986 growing season.

Trends in quantity and diversity of aquatic macrophytes observed during the 2 years (1985 and 1986) of poststocking sampling will be monitored in 1987 to determine if higher stocking rates or integrated control using herbicides is required to achieve more complete watermilfoil control.

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INTEGRATED CONTROL
TECHNOLOGY

Influence of Herbicides on Insects
Used for Control of Waterhyacinths

by
D. C. Pellessier* and A. F. Cofrancesco*

INTRODUCTION

Waterhyacinth (*Eichhornia crassipes* (Martius) Solms-Laubach), one of the world's leading and most damaging aquatic weeds (Holm et al. 1977, Goyer and Stark 1984), is believed to be native to Brazil (Penfound and Earle 1948, Godfrey and Wooten 1979,). A member of the Pontederiaceae family, this free-floating, mat-forming perennial now infests nearly all tropical and subtropical regions of the world (Penfound and Earle 1948, Little 1965).

Its introduction into the United States is linked to the Cotton Centennial Exposition in New Orleans in 1884, although Penfound and Earle (1948) mentioned evidence of waterhyacinth cultivation as a greenhouse and landscape exotic following the Civil War. By 1897, waterhyacinth infestations in the United States created serious problems in navigable waters of the South (Webber 1897, Zeiger 1962). Today it is found in the southern Unted States from South Carolina to Texas and in California (Sanders, Theriot, and Perfetti 1985).

Although beneficial uses of waterhyacinth have been well documented (Bock 1970; Boyd 1968; Hauser 1984; Wolverton 1975; Wolverton and McDonald 1975a, b, c, Wolverton, Barlow, and McDonald 1975), its prolific sexual and reproductive nature makes it noxious in most aquatic systems (Bock 1970, Center and Spencer 1981, Penfound and Earle 1948). According to Westlake (1963) and Bock (1969). *E. crassipes* appears to be among the most productive of photosynthetic organisms. Waterhyacinth infestations hinder navigation, obstruct drainage, destroy wildlife resources, deter outdoor recreation, and purvey hazard to life.

According to Seabrook (1962), infestations of floating plants protect certain mosquito larvae such as the surface-feeding larvae of the malarial vector *Anopheles quadrimaculatus* from predators. Waterhyacinths are also the habitat for many other disease-bearing and pest mosquitos and make larvicide treatment difficult and costly (Seabrook 1962).

Chemical parameters of water quality are altered by the presence of waterhyacinths mats. Lynch et al. (1947) reported absences of oxygen under roots and in the open spaces between the mats and reduced dissolved oxygen levels around the outer edges of the mats. Penfound and Earle (1948) substantiated these findings. In addition, Lynch et al. (1947) found uniform surface temperatures, high carbon dioxide tension, and acidic water in and around the mats.

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Another detrimental effect on aquatic systems caused by waterhyacinth is the role it plays in accelerating eutrophication. In dense populations of waterhyacinth, the loss of water through evapotranspiration is 3.2 (Penfound and Earle 1948) to 3.7 (Timmer and Weldon 1967) times greater than through evaporation alone. Continual decay of waterhyacinth plants aids in the gradual silting of water bodies by building up a residue on the bottom (Timmer and Weldon 1967). Also, waterhyacinth mats in lentic water systems are usually transformed into floating prairies of terrestrial, wetland, and aquatic plants, building downward until they reach the bottom (Penfound and Earle 1948).

The many detrimental effects of waterhyacinth on aquatic systems, along with its estimated relative growth rate of 5 to 6 percent per day (Center and Spencer 1981), suggest the need for effective control. The US Army Corps of Engineers has been involved in waterhyacinth control in navigable waters since before 1897 (Zeiger 1962). The first attempt at control was through the use of log barriers to prevent downstream movement (Zeiger 1962) and a crusher boat to remove the mats (Wunderlich 1962; Sanders, Theriot, and Perfetti 1985). Unfortunately, the lack of knowledge of waterhyacinth growth patterns and optimal harvesting times, together with the slow progress of the boat, resulted in little impact to the infestation (Wunderlich 1962).

From 1902 and 1937, greater success in controlling the weeds was achieved through the use of sodium arsenite (Wunderlich 1962). This chemical control agent was abandoned due to its adverse environmental effects, including its toxicity to humans (Zeiger 1962).

The Kenny, a self-propelled mechanical crusher capable of eliminating 210 acres of waterhyacinth per month, was employed continuously from 1937 to 1951 (Wunderlich 1962; Sanders, Theriot, and Perfetti 1985). Auxiliary conveyors and smaller mechanical harvesters (e.g., saw boats) were used in shallow waters where the Kenny could not be used. The seasonal use of the Kenny in conjunction with the auxiliary conveyors and harvesters resulted in opening hundreds of miles of infested Louisiana waterways (Sanders, Theriot, and Perfetti 1985). Although immediate in its effectiveness, mechanical harvesting was too slow to keep pace with the rapid growth of this aquatic weed (Zeiger 1962).

The predominant method for waterhyacinth control since 1950 has been the use of chemical herbicides, especially 2,4-D (2,4-dichlorophenoxyacetic acid) (Sanders, Theriot, and Perfetti 1985). The seasonal reapplication requirements of herbicides make them expensive to use and effective only as short-term management agents. The primary herbicides employed today include 2,4-D, diquat, and glyphosate (Haag 1986b).

These herbicides are believed to be relatively safe in the concentrations used for waterhyacinth control, although this remains questionable. Among possible side effects are oxygen depletions and resulting fish kills caused by rapid decomposition of massive amounts of plant material (Patton and Starnes 1970) and possible pollution of groundwater supplies. (Haag 1986a).

Biological control offered a possible long-term, low-cost remedy to the water-hyacinth management problem (Perkins 1973a). In the 1960's, the US Department

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of Agriculture began investigating South American insects that feed on water-hyacinth as potential biocontrol agents (Bennett and Zwolfer 1968, DeLoach and Cordo 1976a, Perkins 1974). This research, funded by the Corps of Engineers, led to the release of three exotic insects including two species of waterhyacinth weevils (*Neochetina eichhorniae* (Warner 1970) and *N. bruchi* (Hustache 1928), Coleoptera: Curculionidae) and the waterhyacinth month (*Sameodes albiguttalis* (Warren), Lepidoptera: Pyralidae) (Perkins 1973b; Perkins and Maddox 1976; Center and Durden 1981; Center 1982; Center, Durden, and Corman 1984; Sanders, Theriot, and Perfetti 1985).

These waterhyacinth weevils are semiaquatic, holometabolous members of the tribe Bagoini which undergo development in and feed on various parts of the waterhyacinth plant almost exclusively (DeLoach 1975, 1976; DeLoach and Cordo 1976a, b; Perkins and Maddox 1976). Weevil damage to individual plants and the effects of the damage on waterhyacinth populations have been well documented (Perkins 1974; Forno 1981; Goyer and Stark, 1984; Cofrancesco, Stewart, and Sanders 1985; Sanders, Theriot, and Perfetti 1985).

Typically, biocontrol of aquatic weeds results in a gradual decline of the target species, which frequently is not rapid enough for heavily used waterways (Center, Steward, and Bruner 1982). In high-access areas, rapid reduction of waterhyacinth infestations is achieved through the use of herbicide application; however, the weevil population is reduced due to the abrupt loss of immature and nonmigrating individuals, habitat (Haag 1986a), and food source (Center, Durden, and Corman 1982). Effective biological control results from a dynamic equilibrium between target weed and biocontrol agent (Wright and Center 1984). Population declines of the target weed and its biocontrol agent caused by repeated chemical application precludes reestablishment of the dynamic equilibrium because the population regrowth of the target weed grossly exceeds that of its agent (Center, Durden, and Corman 1982).

Effective, low-cost, long-term, safe waterhyacinth control is still the management ideal for many state and Federal agencies. Accordingly, in an attempt to achieve these goals, research is being directed toward the integrated use of herbicides and biocontrol agents.

In addition to the aforementioned impact of herbicides on weevil populations, there may be other less conspicuous repercussions. In a series of integrated pest management studies, Haag (1986a, b) found that the waterhyacinth weevils do not die as a direct result of exposure to 2,4-D, diquat, glyphosate, or their surfactants and that adult weevils migrate away from declining, sprayed plants toward healthy waterhyacinth plants. Although direct exposure to these herbicides is not lethal to adult weevils, the effects of the reproductive abilities of adults ingesting treated plant material and the population size of subsequent generations have not been thoroughly examined.

Buckingham and Passoa (1985) and Haag (1986b) found evidence that the use of herbicides on waterhyacinth may directly or indirectly affect flight muscle development in *Neochetina*. Haag proposed that an environmental cue such as

herbicide exposure or a decline in plant quality (as seen following herbicide application) may trigger flight muscle generation.

Research on waterhyacinth control indicates that more information is needed on the interactions that occur between chemical and biological control techniques. Understanding these interactions will allow more efficient utilization of resources and a reduction in cost.

PURPOSE AND OBJECTIVES

The purpose of this study is to develop management strategies that will employ chemical and biological agents to gain rapid control of waterhyacinth with long-term effects. The objectives are to:

- Reaffirm that the weevils migrate over short distances from herbicide-treated plants to untreated plants.
- Compare the reproductive capabilities of insects that have been subjected to herbicide applications with control populations.
- Evaluate the wing muscle development in control populations of *Neochetina* and in populations subjected to herbicide applications.
- Develop management strategies utilizing chemicals and biological agents for control of waterhyacinth.

APPROACH

Study I

Since migration of weevils over short distances was observed both in the field and in aquaria (Haag 1986b), it is necessary to prove whether similar behavioral responses are attainable under the conditions of this study. Specific points of this study are to:

- Determine whether the waterhyacinth weevils (*N. eichhorniae* and/or *N. bruchi*) feed on plants treated with 2,4-D, glyphosate, or dequat when offered healthy, unsprayed plants.
- Determine whether the waterhyacinth weevils will migrate to clean plants.
- Determine the amount of time required for each species to migrate and begin feeding on clean (unsprayed, weevil-free) plants.
- Determine the number of weevils of each species that successfully migrate to the clean plants.

Study II

Study II will examine the impact of herbicides on the reproductive capabilities of the two weevils species. Specific objectives of this study are to:

- Determine if adult *N. eichhorniae* and *N. bruchi* exposed to the three herbicides produce the same number of eggs as do unexposed adults of the same species.
- Determine if the progeny of exposed and unexposed conspecific adults are equally capable of eclosion.

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- Determine if the response to herbicide exposure (as measured through fecundity and the production of viable larvae) is equal between the two weevil species.

Study III

This study will examine the influences that herbicides have on flight muscle generation. Specific objectives of this study are to:

- Determine if there are significant differences (in size and numbers) between flight muscles generated by unexposed adult *N. eichhorniae* and *N. bruchi* ingesting deteriorating, herbicide-treated waterhyacinth plant material and those generated by conspecific unexposed adults feeding on healthy, untreated waterhyacinth plant material.
- Determine if *N. eichhorniae* and/or *N. bruchi* adults directly exposed to the herbicide but feeding on healthy, unsprayed plant material exhibit differences in flight muscle development from conspecific, unexposed adult weevils feeding on similar plant material.
- Determine if flight muscle generation by adult *Neochetina* of both species directly exposed to and ingesting plant material sprayed with the three herbicides differs from flight muscle generation by unexposed conspecific adults feeding on healthy, unsprayed plant material.
- Attempt to discern if the effects (if any) on flight muscle development by directly exposing adult *Neochetina* to herbicides differ from the effects on flight muscle generation attained through ingestion of deteriorating, herbicide-treated plant material by cross-comparing data collected from the experimental conditions.

Study IV

Data from Studies I-III will be consolidated and developed into management strategies that will be evaluated under small-scale field testing.

DISCUSSION

Information pertinent to development strategies for effective integration of chemical and biological control of waterhyacinth will be acquired through the series of studies listed above. Data obtained from Study I will determine if weevil responses to herbicide exposure shown by Haag (1986a, b) are also observable under greenhouse conditions. Study I will also provide baseline information on feeding and migration responses of weevils subjected to herbicides.

Study II is important for the development of management strategies because it examines weevil reproductive capabilities. If waterhyacinth weevil reproductive capabilities are affected by herbicide exposure, then population sizes will also be affected. Other considerations in management design are the effects of the various herbicides on the reproductive capabilities of the individual species since the two species may be affected differently by each of the herbicides.

Study III is designed to generate data for improved understanding of the effects of 2,4-D, glyphosate, diquat, and their surfactants on flight muscle development. It should determine whether direct exposure to these herbicides, ingestion of declining plant material treated with them, or both ingestion of (sprayed) declining

plant material and direct exposure stimulate flight muscle generation in either or both species of *Neochetina*. If flight muscles are generated under any of the above conditions, studies should be conducted to determine the effects on weevil population densities in the field.

By analyzing and consolidating the information from the above studies with ongoing studies of other researchers, management techniques will be developed and tested in the field as indicated in Study IV. The data acquired through this research will result in the implementation of management strategies that should finally provide long-term, low-cost, control of waterhyacinth in many types of waterways.

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SIMULATION CAPABILITIES

Computer Simulation Procedures for Aquatic Plant Control

by

Harold W. West*

INTRODUCTION

Computer simulation procedures are being developed under the Aquatic Plant Control Research Program (APCRP) to aid in research and operational control programs involving chemical, mechanical, biological, and physical control methods. Computer simulation models provide systematic methods for determining the effectiveness, cost, and environmental effects of a specific aquatic plant control technique.

Simulation models can be used most effectively to play "what if" games. For example, the US Army Engineer Waterways Experiment Station (WES) has developed two simulation models (HARVEST and STOCK) that can provide information on harvesting rates, costs of various mechanical harvesting systems, and stocking rates for different size classes of white amur for control of submersed plants. These personal computer-PC based codes are based on extensive field and laboratory studies. The WES conceptual framework for developing these procedures is shown as Figure 1.
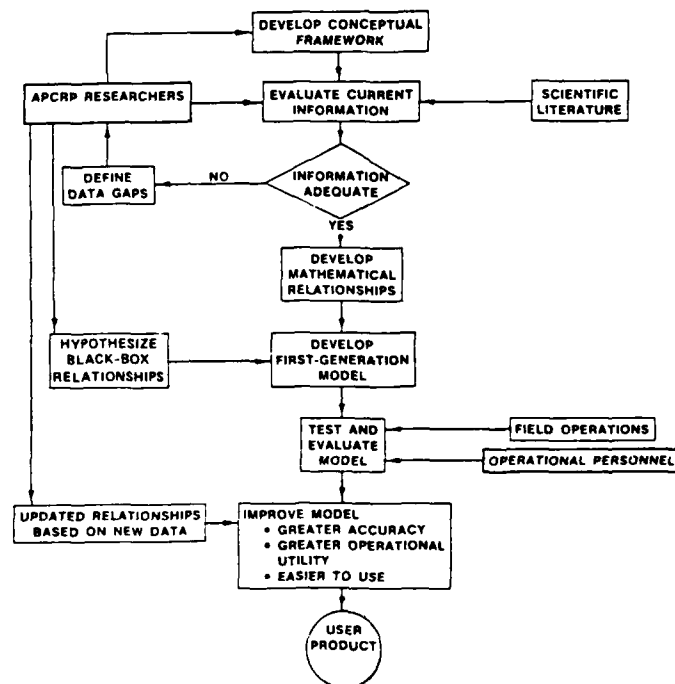
Figure 1. Framework for developing aquatic plant control
simulation models

* US Army Engineer Waterways Experiment Station, Vicksburg, Mississippi.

Work is under way to improve the HARVEST and STOCK models to allow simulations/predictions for additional plant species and other operational control requirements, and to develop new simulation capabilities including biological and chemical control methods.

CHEMICAL CONTROL SIMULATIONS

The initial work related to development of computer simulation models for chemical control has considered the herbicide 2,4-D and waterhyacinths. Control by this method has been operational for many years, and extensive data and relationships are available for analytical model development. During FY 86, WES developed a first-generation code (HERBTRAN) for simulation of chemical residue transport through the water body. The user selects the water surface locations (grid coordinates) where a selected chemical (2,4-D, diquat, fluridone, and endothall) is placed (sprayed) in the water body. The model then calculates the dispersion of the chemical throughout the water body as a function of time, based on chemical half-life decay and water steady-state hydrodynamic flows. Figure 2 shows the half-life decay curve used by the model for 2,4-D butoxyethylester (BEE) at pH 7. Figures 3a and 4a show the areas of the water body treated with 2,4-D (BEE) using a surface loading rate of 40 lb/acre. Example output of the model is shown in Figures 3 and 4. Figure 3 (b, c, and d) portrays the dispersion for different minimum target concentrations of 2,4-D (BEE) 14 days after application. Figure 4 (b, c, and d) portrays dispersion 30 days following application.

During FY 87, North Texas State University, Denton, Tex., under contract to WES, will develop a first-generation simulation model for simulation/prediction of the effects of the herbicide 2,4-D on waterhyacinths. The model will be structured to allow simulation/prediction for a 24-hr time period and will emphasize the interrelation of chemical elements and various impacting attributes
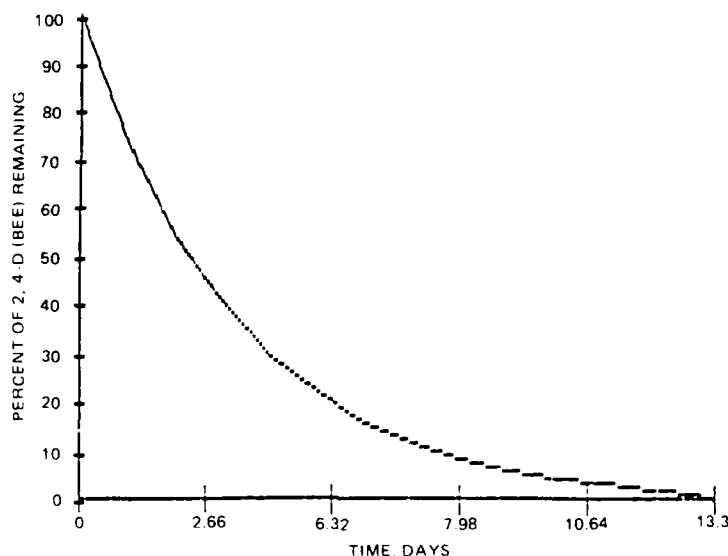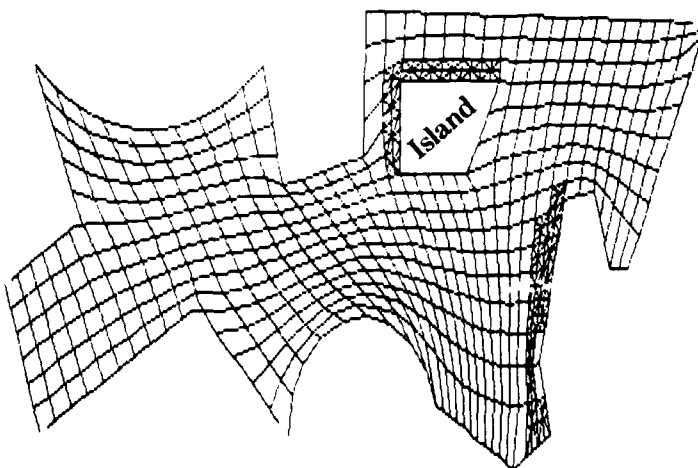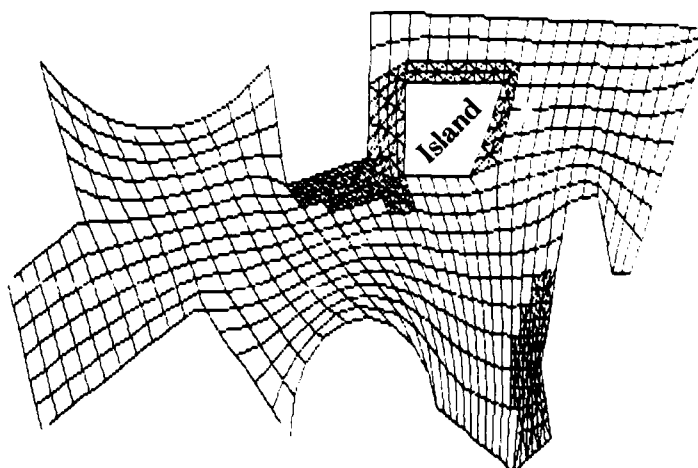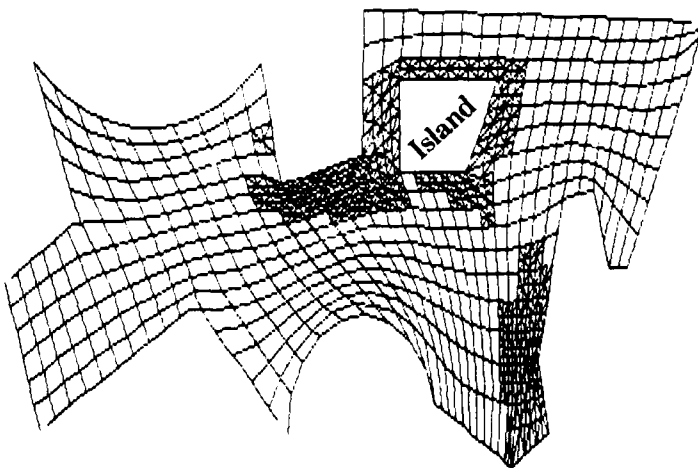

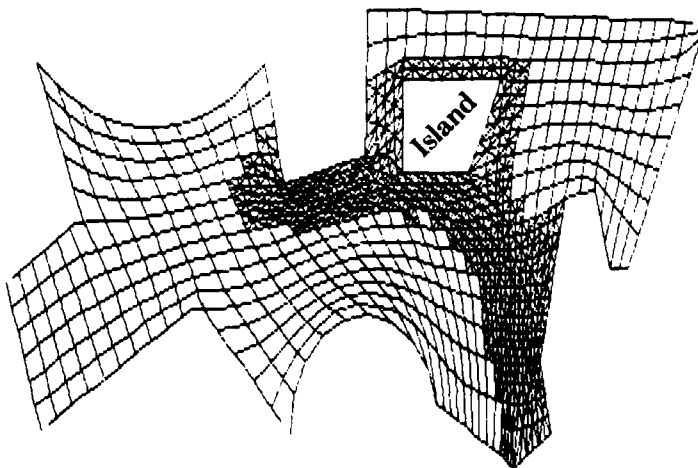Figure 2. Half-life decay curve for 2,4-D (BEE) at a water pH of 7.0

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a. *Areas of herbicide application. Initial (T = 0) concentrations ranged from 0.45 to 2.21 mg/ℓ*

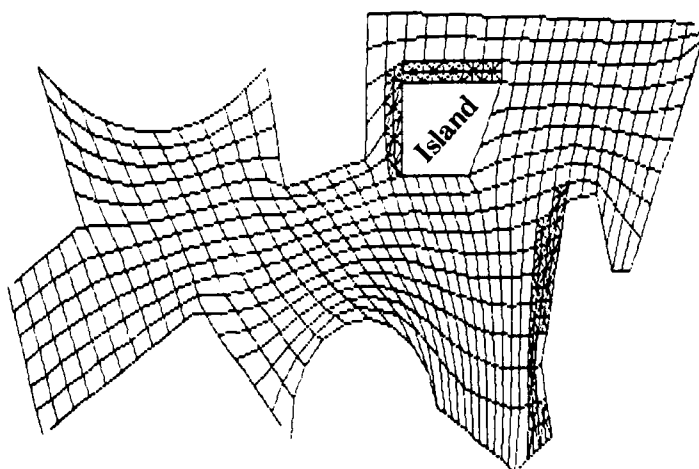b. *Locations where predicted 2,4-D concentrations exceeded 1.10 ppb at T = 14 days*

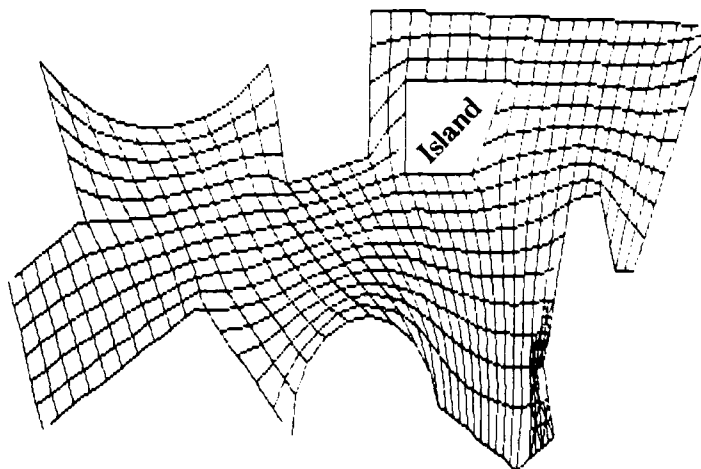c. *Locations where predicted 2,4-D concentrations exceeded 0.11 ppb at T = 14 days*

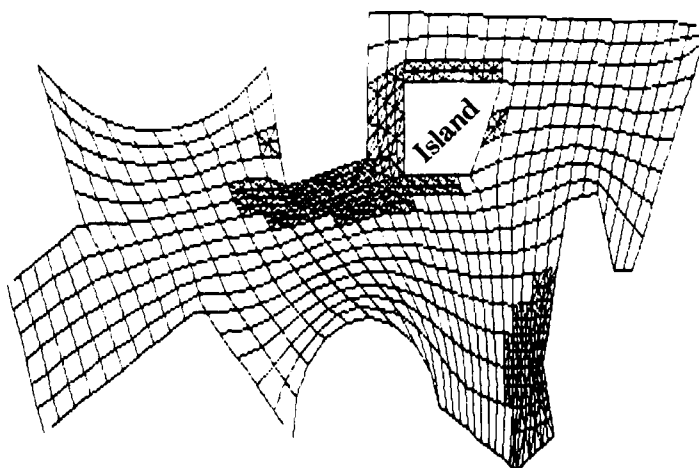d. *Locations where predicted 2,4-D concentrations exceeded 0.01 ppb at T = 14 days*

Figure 3. Computer-generated coordinate system for Houston County Lake, Texas. (Shaded grids in illustration 3a identify locations where 2,4-D was applied in the simulation at a rate of 40 lb/acre; in illustrations 3b, c, and d, shaded grids show the predicted dispersion of 2,4-D within the lake at 14 days postapplication.)
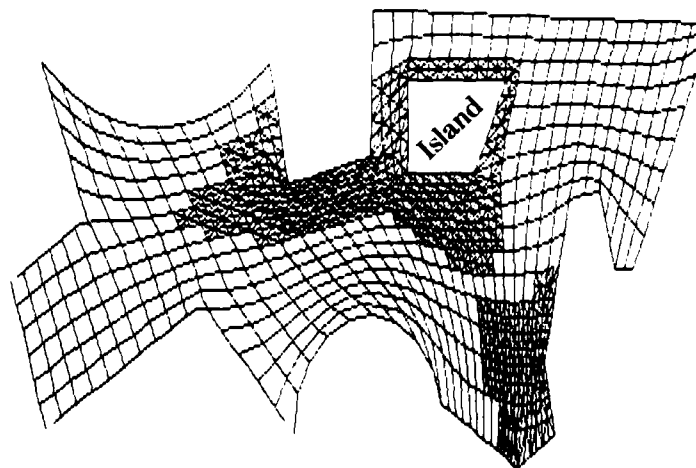
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a. *Areas of herbicide application. Initial (T = 0) concentrations ranged from 0.45 to 2.21 mg/l*

b. *Locations where predicted 2,4-D concentrations exceeded 0.11 ppb at T = 14 days*

c. *Locations where predicted 2,4-D concentrations exceeded 0.01 ppb at T = 14 days*

d. *Locations where predicted 2,4-D concentrations exceeded 0.001 ppb at T = 14 days*

Figure 4. Computer-generated coordinate system for Houston County Lake, Texas. (Shaded grids in illustration 4a identify locations where 2,4-D was applied in the simulation at a rate of 40 lb/acre; illustrations 4b, c, and d, shaded grids show the predicted dispersion of 2,4-D within the lake at 30 days postapplication.)

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of the waterhyacinths. Additional work is also planned on HERBTRAN to allow for the effects of plant infestations within the water body and local weather conditions. Simulation algorithms for river system infestations will also be developed.

BIOLOGICAL CONTROL SIMULATIONS

During the past 2 years, the University of Southern Mississippi, Hattiesburg, Miss., under contract to WES, developed a first-generation simulation capability for control of waterhyacinths by two species of *Neochetina* weevils (*N. bruchi* and *N. eichhorniae*). This model (INSECT) is briefly described in the following paper by Drs. Howell, Wooten, and Akbay, entitled "INSECT: A Computer-Aided Management Tool for Prediction of Biocontrol Effectiveness."

FUTURE SIMULATION DEVELOPMENTS

WES plans to continue development of computer-simulation models in support of the APCRP. The development schedule will depend upon results of research being accomplished in the various APCRP technology areas, such as chemical, biological, ecology, and integrated. The following simulation models are tentatively planned.

a. Chemical/plant systems.
    (1) Diquat/waterhyacinths.
    (2) Diquat/waterlettuce.
    (3) 2,4-D BEE/Eurasian watermilfoil.

b. Biological/plant systems.
    (1) *Sameodes* (moth)/waterhyacinths.
    (2) *Neohydronomus* (weevil)/waterlettuce.
    (3) *Namangana* (moth)/waterlettuce.

INSECT: A Computer-Aided Management Tool for Prediction of Biocontrol Effectiveness

by
Fred G. Howell,* Jean W. Wooten,* and Kunter S. Akbay**

INTRODUCTION

The Aquatic Plant Control Research Program is being managed by the US Army Corps of Engineers Waterways Experiment Station and is currently conducting research to produce computer-oriented tools for the development of biocontrol strategies for waterhyacinth and other aquatic plants. This research will yield a computer model for predicting the interactions of aquatic plants such as waterhyacinth (*Eichhornia crassipes* (Mart.) Solms) and waterlettuce (*Pistia*) and various insects (*Neochetina* spp., *Sameodes albiguttalis*, and *Neohydronomus* sp.) that have been imported for control purposes. INSECT, a first-generation model for waterhyacinth and *Neochetina* spp., is under development. The purpose of the paper is to present a brief overview of INSECT and to illustrate its intended future use for predicting biocontrol effectiveness.

OVERVIEW OF INSECT

Description

INSECT is a user-oriented software package written in FORTRAN and developed for use with personal computers (a math coprocessor is required). The model currently includes components for the growth and development of waterhyacinth, its biocontrol insects, and interactions between waterhyacinth and these insects. The waterhyacinth module can be operated independently or with the weevil module.

INSECT is "dynamic" and provides predictive data on a daily basis under local weather conditions (daily minimum and maximum temperatures and solar radiation). Daily values (on a square metre basis) are calculated for several plant and insect variables, including kilograms of plant biomass, number of plants, number of leaves, amount of detritus, and numbers of eggs, larvae, pupae, and adults. Plant loss due to weevil activity is based upon assumptions of meristematic damage by third instar larvae.

The plant module was designed after careful study of the techniques of Ewel, Braat, and Stevens (1975); Mitsch (1975, 1976); Vega (1978); Lorber, Mishow, and Reddy (1984); and others. For the study reported here, a nonlinear relationship (with light and temperature as independent variables) derived from the results of the above-named and other studies was used. Thus, the module is a derivation

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of a deterministic procedure. Furthermore, because energy balance equations are difficult to handle (partly because required driver data are not usually easily obtained), the procedure chosen to produce simulations of cumulative biomass versus time is reasonable at this development level of the model. Indeed, it is probably the only applicable approach given the present dearth of appropriate data.

The insect module was designed after Brown, McClendon, and Jones' module (1982). Algorithms to simulate population dynamics of *Neochetina* are based upon biological and ecological information found in DeLoach and Cordo (1976a, b), Center and Spencer (1981), Stark and Goyer (1983), Center and Durden (1986), Center and Spencer (unpublished manuscript*), and others. Development of weevils is accomplished via accumulation of physiological time in day-degrees based upon average daily temperatures. Development rates, fecundity, survivorships and mortalities, and other features controlling the insect populations were based on the works of the above authors. Due to differences in the developmental times and oviposition rates between *Neochetina bruchi* and *N. eichhorniae*, the weevil module is composed in logic of two subroutines which differ mainly with respect to these two variables. As with plants, more complexities can and shall be added to the module as more definitive information becomes available. As the module currently operates, realistic dynamics of the insect populations are reflected in simulations (Akbay, Wooten, and Howell, in preparation).

A flowchart of the model INSECT is shown in Figure 1. After the plant and insect components of the model are initialized on the first day of simulation, an iterative logic simulates the aquatic plant ecosystem. First, on each simulation day, daily weather data are read. Then the plant module is called to calculate the total biomass available to insects. The weevil module is called, and total numbers of each life stage for each weevil species are calculated. After the plant and weevil modules are called, the impact on waterhyacinths by *Neochetina* spp. is calculated and the daily results are output.
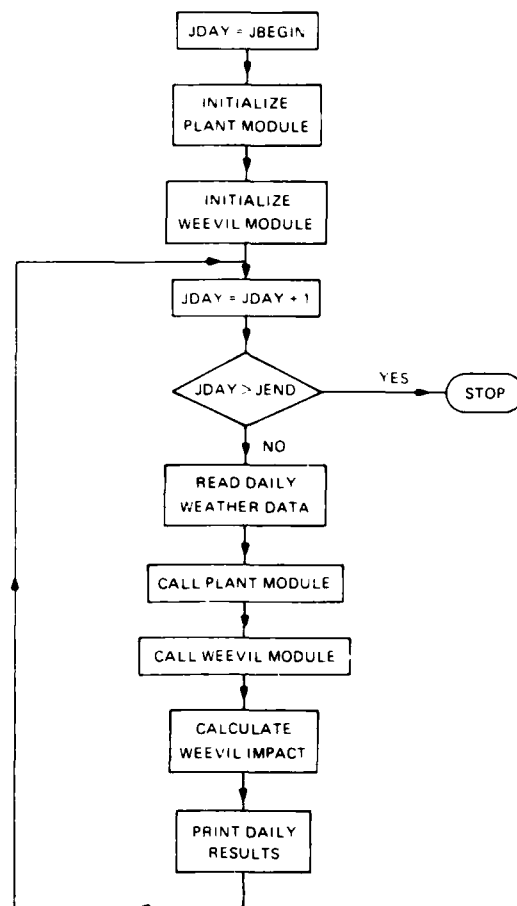
INSECT can operate between any two user-defined starting and ending Julian dates, up to 3 years in duration. Run time is about 4 min per simulated year (on an IBM-AT). User-friendly "prompts" encourage the operator for input information. Results can be plotted directly to the screen; a dot-matrix printer can be used for hardcopy products of screen graphics.

Utility

INSECT has been developed to assist plant control managers in effectively using *Neochetina* spp. to control waterhyacinths. Other biocontrol insects and plants are

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* "Herbivore Induced Alteration of a Marsh Community: Waterhyacinth and Weevils."

JDAY = JBEGIN

INITIALIZE
PLANT MODULE

INITIALIZE
WEEVIL MODULE

JDAY = JDAY + 1

JDAY > JEND

YES → STOP

NO

READ DAILY
WEATHER DATA

CALL PLANT MODULE

CALL WEEVIL MODULE

CALCULATE
WEEVIL IMPACT

PRINT DAILY
RESULTS

Figure 1. Flowchart of INSECT model

being modeled and will eventually be included in INSECT. However, this first-generation version considers only waterhyacinth and two insects. Using this model, it is possible to predict for a potential or present waterhyacinth problem. Thus, the model will:

- Predict waterhyacinth growth in the absence of biological control agents and thereby establish a data base for plant biomass under defined environmental conditions.

- Predict insect densities over a maximum of 3 years.

- Predict the impact of insects on the growth of waterhyacinths during the same time period.

- Demonstrate the overall effectiveness of a proposed biocontrol strategy.

Input

To perform a simulation using INSECT, site-specific information must be collected. Technically, this information could be gathered anytime during the year, but we suggest that this be done during winter months while the site is not undergoing flux. (Eggs, for example, are virtually impossible to count accurately during the growing season. Assessments made during the early winter while the populations are less reproductive will yield more accurate information for initiating simulations for the site.) Initial information should include the following:

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- *Estimation of current waterhyacinth biomass.* Plant data can be collected as either wet or dry weight, but must be input to the model as kilograms per square metre. If the data are collected as wet weight, a factor of 0.05 is used to convert to dry weight.

- *Estimation of current insect population.* These data should be input (for each insect species present) in numbers of larvae, pupae, and adults per square metre. If known, the number of eggs can be input as well.

- *Julian date of collection of above information.* Because the model makes its calculations on a daily basis and timing of insect development is important, input values should be consistent with the date of data collection.

- *Weather data.* Dynamics of both plant and insect populations are partially regulated by daily weather (temperatures and solar radiation); the model, therefore, builds a temporary weather data file specifically for a simulation. Since daily weather is difficult to predict, the current version of the model contains several historical weather data files of actual data recorded from geographical regions, where waterhyacinths grow (one weather data file = 1 year of historical data). In initiating the model, a user must select (from a menu) which one (or ones) of several historical weather data files to use. The model will then create a temporary weather data file to be used in the calculations for that simulation.

Output

With each simulation, the model outputs two data files: one for plants and one for insects. Data contained in each file are accessible to the user by way of screen plots. Currently, the user may choose plant biomass, plant numbers, or density values for any one of the four life stages of the insect(s). As stated earlier, these plots will first appear on the screen and can be output to hardcopy product by way of the "print screen" function from the computer keyboard. Additionally, programs are being developed that will enable the user to analyze each data file.

DEMONSTRATION OF MODEL USE

INSECT simulates for only one set of initial site conditions per run. Therefore, some user forethought is needed to realize the potential of the model. Such a plan would likely revolve around "what if" questions, i.e., what would plant biomass be for any given Julian date during the next 3 years if x numbers of insects were present in early winter of year 1?

As an example, the user would first gather information on the amount of plant biomass and the number of insects (if present) for the site under question. He or she would then select which three files of weather data to use in the simulations. (In the following example, three different files of weather data were used, i.e., each weather data file was different from the others.) Next, assuming an initial plant biomass (dry weight) of 0.769 kg/m^2 on Julian Day 1, the user might then make the following simulations to determine waterhyacinth productivity under the following conditions:

- *Simulation 1* — without insects (to establish a baseline, or control, data base).
- *Simulation 2* — with insects present at low levels at the site (example: 11

larvae, 31 pupae, and 11 adults of *N. eichhorniae* per square metre present on Julian Day 1.

- *Simulation 3* — with insects present in high levels (example: 40 larvae, 50 pupae, and 40 adults of *N. eichhorniae* per square metre on Julian Day 1).

Figure 2 shows the results of three simulations using the above scenarios of weevil densities and initial plant biomass. Note that although the weather data were different for each simulated year, each simulation was performed under the same 3-year set of weather conditions. The solid line represents the predicted waterhyacinth biomass (control — without biocontrol insects, Simulation 1 above), curve for 3 simulation years; the dashed line traces the model's predictions under Simulation 2 (insects were present in the system at the beginning of the simulation, but in low numbers). The dotted line is a plot of the model's predictions for waterhyacinth biomass under conditions described by Simulation 3 (high density of insects).

Several points regarding the interactions of waterhyacinth, insects, and weather can be illustrated by this example. Weather, for example, has a major impact on the productivity of the plant. This is demonstrated by the differences in total biomass between first, second, and third simulated control years. Also, note that insect impact on the plants is not particularly severe during the first year, regardless of conditions, but impacts increase in subsequent years.

For Simulation 2 or 3, note the timing of the departures in biomass from the simulated control during years 2 and 3. In these simulations, timing of impacts
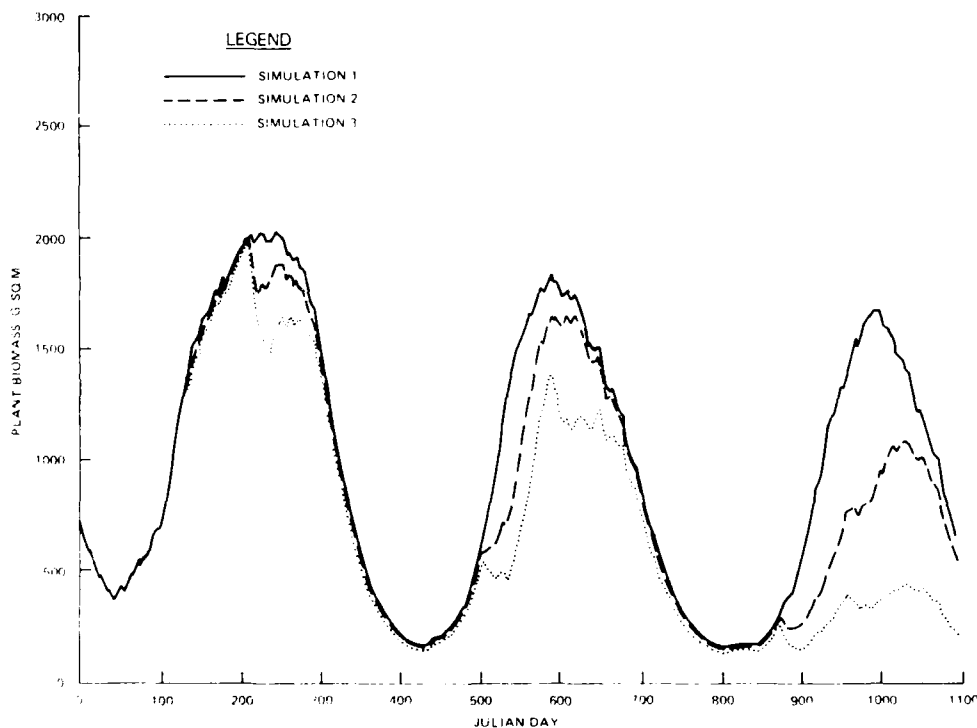

Figure 2. Predicted plant biomass over a 3-year period under different scenarios for initial insect densities

occurs progressively earlier in the growing seasons. Also, the impacts during the early part of the year become more severe with time. In this case, the insect data file generated by the model shows that the numbers of overwintering immatures (larvae and pupae) increased with time. Hence, numbers of immatures present during the early part of the year steadily increased from year to year.

These are important points because obtaining the desired level of control during the first year by introducing insects might require enormous time and effort to achieve the necessary densities of the correct physiological and developmental states. Also, one must be realistic in terms of the numbers of insects that can physically be introduced. To effect the control it would be easier and cheaper to let nature synchronize insect development.

The user might choose to make additional simulations under different conditions depending upon the problem at hand. Since weather obviously influences plar.t productivity and insect development, other simulations could be done using different weather data files or using other starting values for plant biomass and/or insect densities. The major point, however, is that the model will permit a user to predict and evaluate the consequences of specific biocontrol scenarios for a specific site for defined sets of conditions.

MODEL VERIFICATION

Data presented in Figure 2 (Simulation 2) are, in reality, the model's predictions for conditions (weather, initial plant biomass, and initial *N. eichhorniae* densities) recorded by Center in 1976 at Lake Alice, Gainesville, Fla. Beginning numbers of insects and plant biomass are those resulting from the introduction of infected waterhyacinths (approximately 40 larvae and 10 pupae) in two square-metre plot, on Julian Day 51 of the previous year (1975). According to Center's data, *N. eichhorniae* was widely distributed in Lake Alice by the beginning of the next growing season (1976) and in numbers approximate to those used to initiate Simulation 2. The resulting plant biomass curve (dotted line) fits reasonably well within the 95 percent confidence intervals calculated by Center for waterhyacinth biomass during the ensuing 3 years (1976, 1977, and 1978), as did the plots of data for adult and larval densities (not shown). A more detailed discussion of these data and their use in model verification can be found in Akbay, Wooten, and Howell (in preparation).

STATUS OF THE MODEL

Four species of insects and two aquatic weed species are currently being modeled. Also, the US Department of Agriculture is currently collecting data at two different geographical sites in Florida that have both species of *Neochetina*. These data will be used to help validate and refine relationships used in INSECT. The conceptual module for *S. albiguttalis* has been completed, and coding is under way for this species. A literature review and conceptual model for *Neohydronomus*, a biocontrol insect for *Pistia* (waterlettuce), are in progress.

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CHEMICAL CONTROL TECHNOLOGY

Herbicide Concentration/Exposure Time Relationships

by
Howard E. Westerdahl*

BACKGROUND

The objective of this work is to determine the effective range of herbicide concentrations and exposure times that control target aquatic plants. Over the past few years at WES, a diluter system was used to estimate the range of herbicide concentration/exposure times necessary to control watermilfoil (*Myriophyllum spicatum* L.) and hydrilla (*Hydrilla verticillata* Royle). A similar system was used by the US Department of Agriculture (USDA) Aquatic Plant Management Laboratory (APML), Fort Lauderdale, Fla. (under an interagency agreement). Provided below is a listing of herbicides already tested at WES or by the USDA APML, and those remaining to be evaluated (——) at WES.

Herbicide	Watermilfoil	Hydrilla
Diquat	— —	Completed
2,4-D	Completed	NA
Endothall	— —	Completed
Fluridone	Completed	Completed
Dichlobenil	— —	— —
Triclopyr	— —	— —

A random design was used to assign one of five exposures to a specific aquarium. Following exposure, the aquaria were flushed and refilled twice. Weekly evaluations of herbicide effects on the plants were then performed over a 12-week posttreatment period. Finally, design conditions were set to approximate a natural hard-water lake, i.e., water temperature 24° to 26° C; pH 7.6 to 8.0; total hardness 160 mg/l; and photoperiod of 10 hr.

Results to date are summarized in Figures 1-4. Refinements of these relationships may be necessary following field evaluations; however, they should provide developers with the information required to initiate improvement in existing formulations as well as to design new controlled-release formulations. Figure 1 describes diquat efficacy on hydrilla. As long as a diquat formulation (or application technique) can deliver or exceed the minimum concentration/exposure time (shaded area) in a similar aqueous environment, control of hyrilla should be expected. Likewise, this would be assumed for 2,4-D and watermilfoil (Figure 2) and endothall and hydrilla (Figure 3).

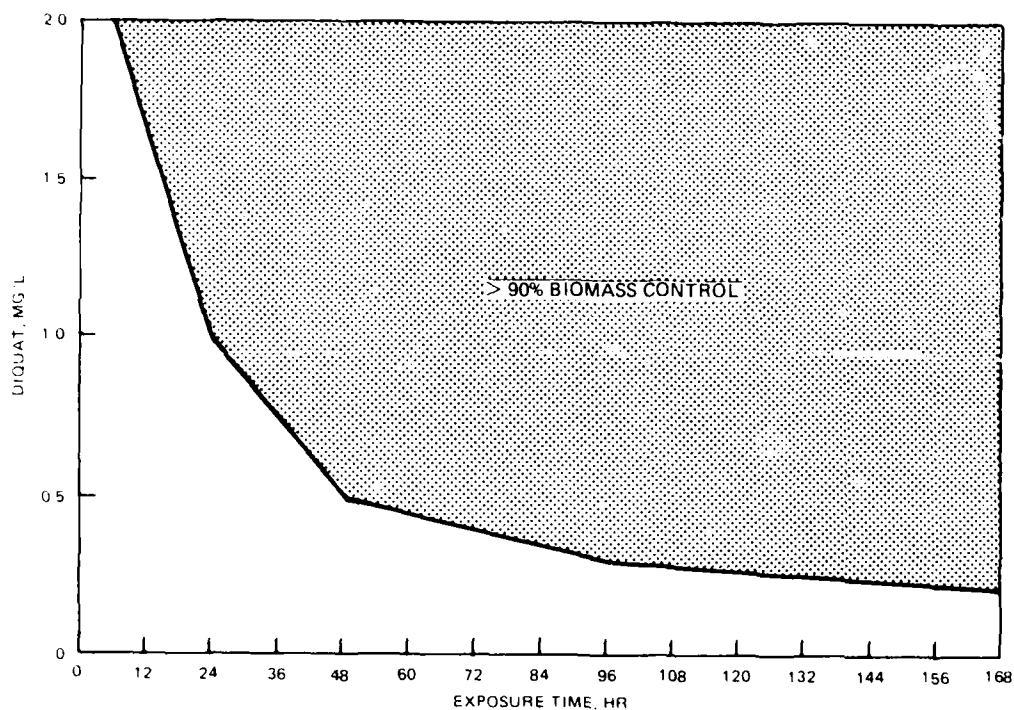* US Army Engineer Waterways Experiment Station, Vicksburg, Mississippi.

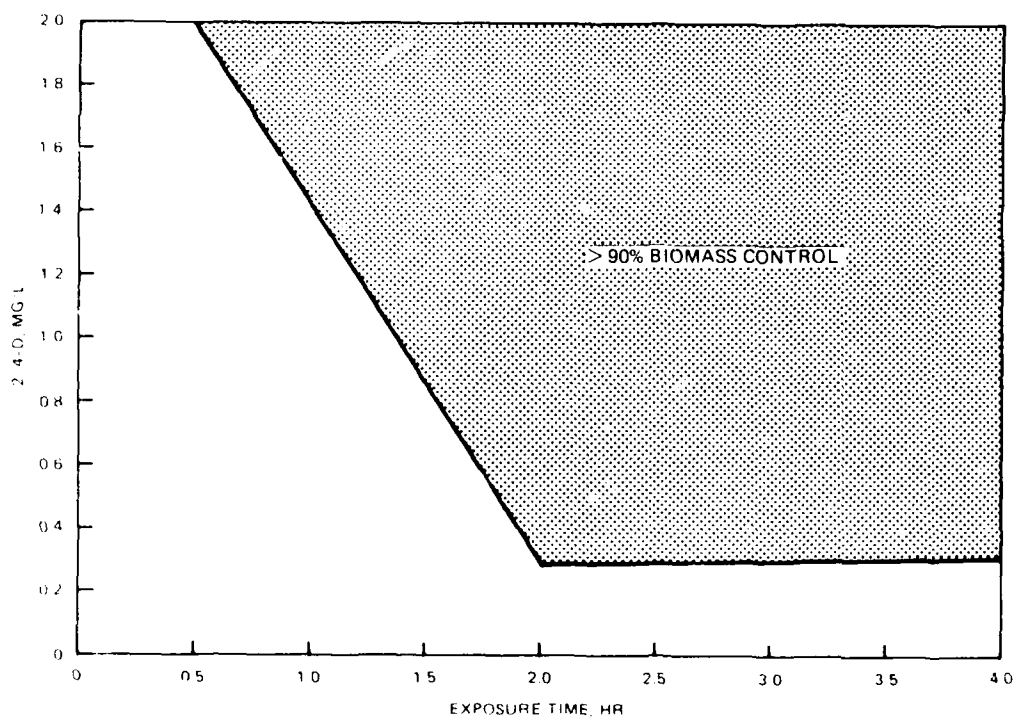Figure 1. Effects of diquat concentration/exposure time on *H. verticillata* (Royle)

Figure 2. Effects of 2,4-D concentration/exposure time on *M. spicatum* L.
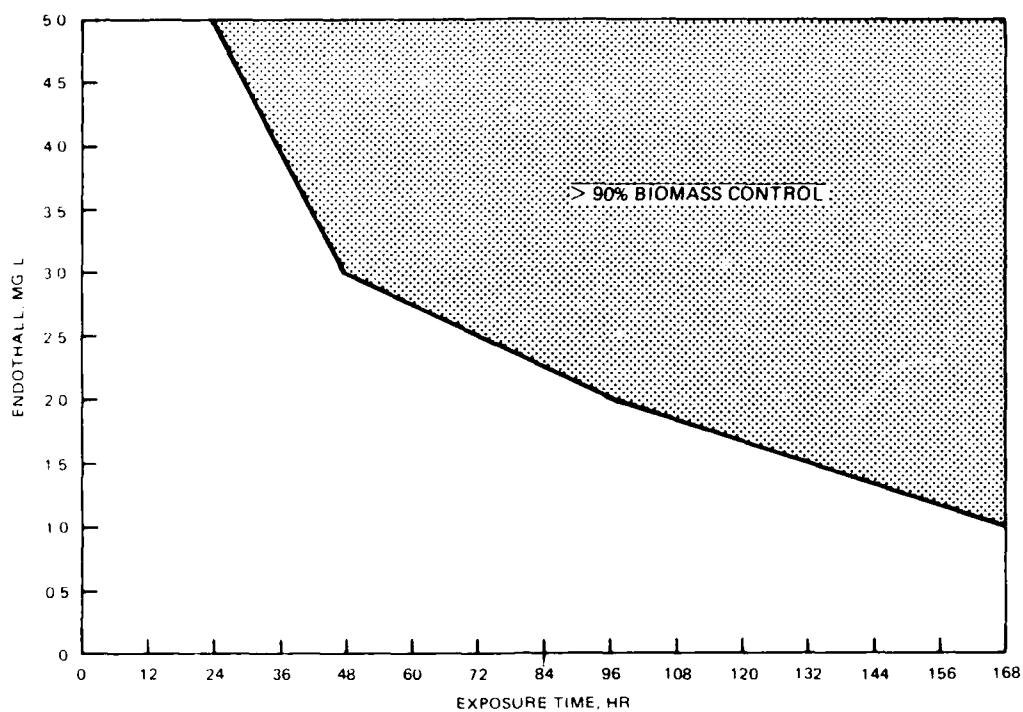
170

Figure 3. Effects on endothall concentration/exposure time on *H. verticillata* (Royle)
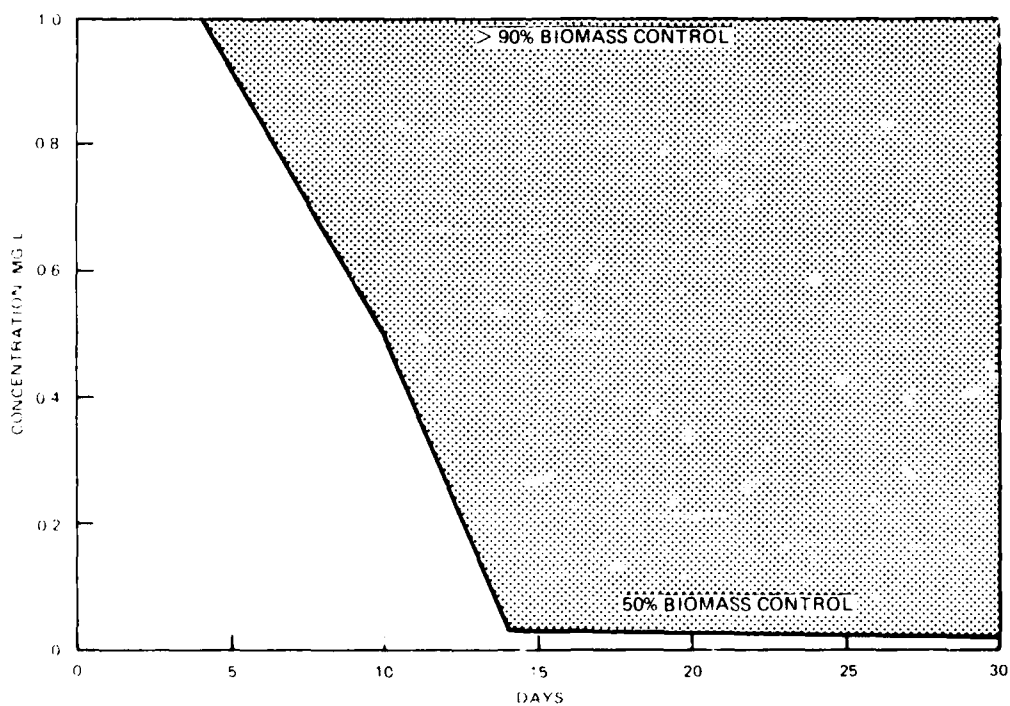

Figure 4. Effects of fluridone concentration/exposure time on *H. verticillata* (Royle)

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Fluridone effects (Figure 4) were considerably different from those obtained with the previously mentioned herbicides. With a lower fluridone concentration (>0.1 mg/l) and longer exposure time (>14 days), an approximate 50-percent reduction of hydrilla biomass was achieved. As the fluridone concentration was increased (up to 1.0 mg/l) and exposure time was decreased from 14 days to a minimum of 4 days, the percent biomass reduction approximated 100 percent. This relationship was not observed for watermilfoil. Only 15 $\mu g/l$ for approximately 20 days exposure or 30 $\mu g/l$ for approximately 12 days was required to reduce watermilfoil biomass by approximately 95 percent. Apparently, watermilfoil is more sensitive to fluridone than is hydrilla.

FUTURE STUDIES

During FY 87-88, verification of the previously described relationship of 2,4-D and watermilfoil will be completed, and similar studies with endothall and diquat will be completed on watermilfoil. During FY 89-90, dichlobenil and triclopyr will be tested.

COOPERATIVE HERBICIDE TESTING WITH INDUSTRY

Over the past year, cooperative field testing of Garlon 3A (triclopyr) with Dow Chemical U.S.A. was initiated under an Experimental Use Permit (EUP) at Lake Seminole, Ga. A similar field study will be conducted by the Bureau of Reclamation, Denver, Colo., in the Northwest during FY 87. The objectives of these studies were to determine the environmental fate and dispersion characteristics of this herbicide when applied to 10-acre plots in reservoirs, representing geographically distinct portions of the United States. Additional dispersion studies in other reservoirs around the country may become necessary if the US Environmental Protection Agency (USEPA) determines that the data are inadequate for representing reservoirs of the southeast and northwest sections of the country. Currently, the WES is awaiting completion of the residue analyses by Dow Chemical to complete the final report and submit it to the USEPA.

A similar study was planned with Uniroyal and the Tennessee Valley Authority to evaluate CASORON 10G (dichlobenil) under an EUP for supporting a potable water tolerance. Unfortunately, the EPA had several questions of the data submitted to support the EUP. The subsequent delay required that the study be considered for initiation in FY 87. It was decided, however, to conduct limited replicated field tests on 1-acre plots in Lake Seminole, Ga., using the USEPA-registered 10G formulation and a new 20G formulation provided by Duphar, Inc., of the Netherlands. Results from these small-scale tests will benefit the final design of the EUP sampling program for FY 87.

Field Evaluations of
Triclopry and Dichlobenil

by
Wm. Reed Green*

INTRODUCTION

Information concerning environmental fate and dispersion of aquatic herbicides is needed for those chemicals being considered for registration by chemical companies and regulatory agencies. During the summer of 1986, the US Army Engineer Waterways Experiment Station conducted two field studies to evaluate the herbicides dichlobenil and triclopyr (Garlon 3A). The first study using dichlobenil was a small-scale field study involving replicated plots of approximately 0.5 ha (1 acre). The second study using Garlon 3A was conducted under an Experimental Use Permit (EUP) using operational-sized 5 ha (10-acre) plots. Both studies were conducted in Lake Seminole, Fla.

Dichlobenil is a registered herbicide produced by Duphar, Inc., and licensed by Uniroyal and PBI, Gordon, Inc., under the trade names CASORON 10G and NOROSAC 10G, respectively. The chemical is an aromatic nitrile compound used as a preemergent, selective herbicide in terrestrial and aquatic environments. The restrictions on its use as an aquatic herbicide limit its use to primarily farm ponds and drainage canals. Consequently, treated water cannot be used for irrigation, livestock, or human consumption. Moreover, fish from treated water cannot be used for 90 days after application. Finally, the herbicide cannot be applied to water containing shellfish. Uniroyal has petitioned the US Environmental Protection Agency for an EUP to permit the collection of environmental fate and dispersion data at selected locations around the United States during the summer of 1987. The data will be used to support the establishment of potable water, fish, and shellfish tolerances. In addition, toxicological data will be collected and submitted to support existing and expanded aquatic use registrations.

Dichlobenil is an inhibitor of germination and of actively dividing meristems.* The site of activity is at growing points, including root tips. Growth inhibition is followed by disruption of meristematic tissue and phloem. Dichlobenil affects both monocotyledonous and dicotyledonous plants and is most successful on germinating seeds and young seedlings. Absorption is from the soil by the root system whereby it is rapidly translocated upward throughout the plant. Dichlobenil poses no adverse effects on wildlife and is not acutely toxic to fish at herbicidal concentrations.

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* US Army Engineer Waterways Experiment Station, Vicksburg, Mississippi.
** Weed Science Society of America. 1983. *Herbicide Handbook*, 5th ed., Champaign, Ill.

The current field study was conducted to provide background information on herbicide residue persistence and sampling frequency for accomplishing the objectives of the field study to be conducted under an EUP during the summer of 1987.

A formulation of the systemic herbicide triclopyr, marketed by DOW Chemical Company under the trade name GARLON 3A, is presently under consideration by DOW for aquatic registration. Triclopyr is a picolinic acid compound used as a selective, postemergent herbicide. Moreover, it is a selective auxin-type herbicide that appears to be similar to that of phenoxy herbicides. It is absorbed through both roots and foliage. It readily translocates throughout and accumulates in meristematic tissue. Triclopyr's half-life in water is 10 hr and characteristically has a low-order toxicity to wildlife and fish.*

Information is needed on triclopyr's efficacy and dissipation characteristics, i.e., environmental fate and dispersion, to properly evaluate its value as an aquatic herbicide and to justify the establishment of potable water, fish. and shellfish tolerances.

Cooperators in these studies were The Center for Aquatic Weeds, University of Florida; the Fisheries Division of Georgia's Game and Fish Department; and DOW Chemical Company.

MATERIALS AND METHODS

Both the dichlobenil and triclopyr field studies were conducted at Lake Seminole, Fla. The sites in which the test plots were laid out were infested primarily with *Myriophyllum spicatum* L. (Eurasian watermilfoil) and *Hydrilla verticillata* Royle (hydrilla). Studies were initiated in late June for dichlobenil and early July for triclopyr.

Dichlobenil study

Tests were conducted in duplicate 0.5-ha plots with two formulations of dichlobenil, i.e., a 10- and 20-percent granular (10G and 20G). A random experimental design with two replicat s was used. Half of the plots were treated with the herbicide endothall (Aquathol) at a rate of 120 kg/acre to achieve 3 mg acid equivalent (ae)/ℓ, by surface injection, 8 days prior to dichlobenil application. The endothall treatment was used to achieve "knockdown" of the standing plant population to assess dichlobenil's capacity for retarding hydrilla regrowth from tubers and stems. This would be somewhat analogous to a spring treatment. Dichlobenil, alone, was tested for comparison with the combined treatment, i.e., with endothall. Once it has been registered and tolerances have been established, dichlobenil would be recommended for use in the spring, when hydrilla tubers break dormancy and start to grow.

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* Weed Science Society of America. 1983. *Herbicide Handbook*, 5th ed., Champaign, Ill.

Four tests were conducted, with one plot left untreated (reference) and one plot treated with endothall only and used as a reference for regrowth comparison with plots treated using 10G and 20G. These six treatments are listed below.

- Reference.
- Endothall only (3 mg/l)—reference.
- Endothall, followed by 10G.
- Endothall, followed by 20G.
- 10G only.
- 20G only.

Both dichlobenil formulations were applied at a rate of 7.5 kg ae/ha (15 lbs ae/acre).

Each plot was divided into quadrants. Samples collected for residue analysis included water and sediment. Water (1.0 l) was collected from the center of each quadrant and composited into one 1.0-l sample. A separate 1.0-l sample was collected from the center of each plot. Sediment samples were collected from the center of each quadrant as well as the center of the plot and composited to provide one residue sample. Samples were collected on the pretreatment day, the treatment day, and posttreatment days 1, 5, 8, 12, 21, 34, and 55. Herbicide efficacy determinations were made on the same schedule, as well as posttreatment days 104 and 177.

Triclopyr study

The plots were 5 ha in area to approximate an operational treatment using triclopyr according to labeled use and recommended application techniques. Hence, environmental fate and dispersion information would approximate actual use conditions. One plot was left untreated and used as a reference, the second plot was treated aerially by helicopter, and the third plot was treated by surface injection using an airboat. Triclopyr was applied at a rate of 15 kg ae/ha.

Each plot was divided into quadrants. Samples for residue analysis included water, sediment, plants, fish, clams, and crawfish. Zooplankton samples were taken for qualitative analysis. Water was collected at two depths where possible, 0.3 m below the surface and 0.6 m above the sediment. Separate water samples (1.0 l) were collected at the center of each quadrant and the center of each plot. Additional water samples were collected approximately 60 m outside the perimeter of each test plot. An additional water sample site was located approximately 1.0 km downstream from the treated areas. Sediment samples (1.0 l) were collected at the center of each quadrant and the center of each plot. Plant samples (grab) were composited from the center of each quadrant, and a separate plant sample was collected from the center of the plot. Fish samples were collected by using boat-mounted electroshocking equipment provided and operated by the State of Georgia, Game and Fish Department. Clams were collected from the indigenous population. Crawfish for residue analysis were introduced and placed in two cages within each plot.

Water quality was monitored continuously using self-contained, submersible Hydrolab water quality samplers (model 2030-DS). Residue sample days included

the pretreatment day, treatment day, and posttreatment days 1, 3, 7, 14, 21, and 42. Herbicide efficacy determiniations were made on the same schedule, as well as posttreatment days 90 and 163.

RESULTS

Presently, results from the residue analysis have not been obtained for either study. However, herbicide efficacy determinations have been qualitatively examined. These determinations have been made through observational evaluations based on pretreatment condition, reference condition, and nontreated areas.

Dichlobenil study

Dichlobenil applied to standing plants (those plots without pretreatment control by endothall) resulted in little, if any, visual control. These plots, along with the reference plot, contained approximately 100-percent cover (0-percent control) throughout the study. In plots that were pretreated with endothall, the vegetative standing crop within the plot was controlled and resting on the sediment surface by the time dichlobenil was applied.

In plots treated with endothall, regrowth started to occur by posttreatment day 21 and continued to increase. However, the 10G and the 20G formulations controlled regrowth within the pretreated endothall plots through posttreatment day 55 (Figures 1 and 2). At day 21, the 20G formulation appeared to provide better control than the 10G formulation. However, by day 55 the total standing crops within these plots were very similar. At this time, *H. verticillata* began to infest the open plots. By day 104, *H. verticillata* increased in abundance in most of these plots and, in one, case, almost totally filled the plot. Vegetative control at this time appeared to be slight if any. By day 107, natural competitive forces produced highly variable plot communities and standing crop. Some plots were dominated by *M. spicatum* while others were dominated by *H. verticillata*. At this time, all plots except one were almost totally covered with vegetation.

Triclopyr study

Efficacy results from the triclopyr study were different from the dichlobenil study. First, triclopyr was applied to the mature population of vegetation. Second, triclopyr, having a phenoxy-type mode of action, has no effect on *H. verticillata*. Since only three 5-ha plots were used, the variability in vegetative structure was greater than desired.

Pretreatment survey of the plots showed that the reference plot was covered almost entirely with *M. spicatum*. The aerially treated plot was about 75-percent covered, primarily by *H. verticillata*, while the surface-treated plot being 80-percent covered contained equal portions of each species (Figure 3). By day 21, both triclopyr-treated plots contained no *M. spicatum*, but the total cover remained the same. *Myriolphyllum spicatum* was replaced by *H. verticillata*. Conditions were unchanged by day 42 in the aerially treated plot. However, by day 42 in the surface-treated plot, *M. spicatum* started to grow back. This reinfestation was reduced again by day 90, both plots without *M. spicatum*. Total cover increased between
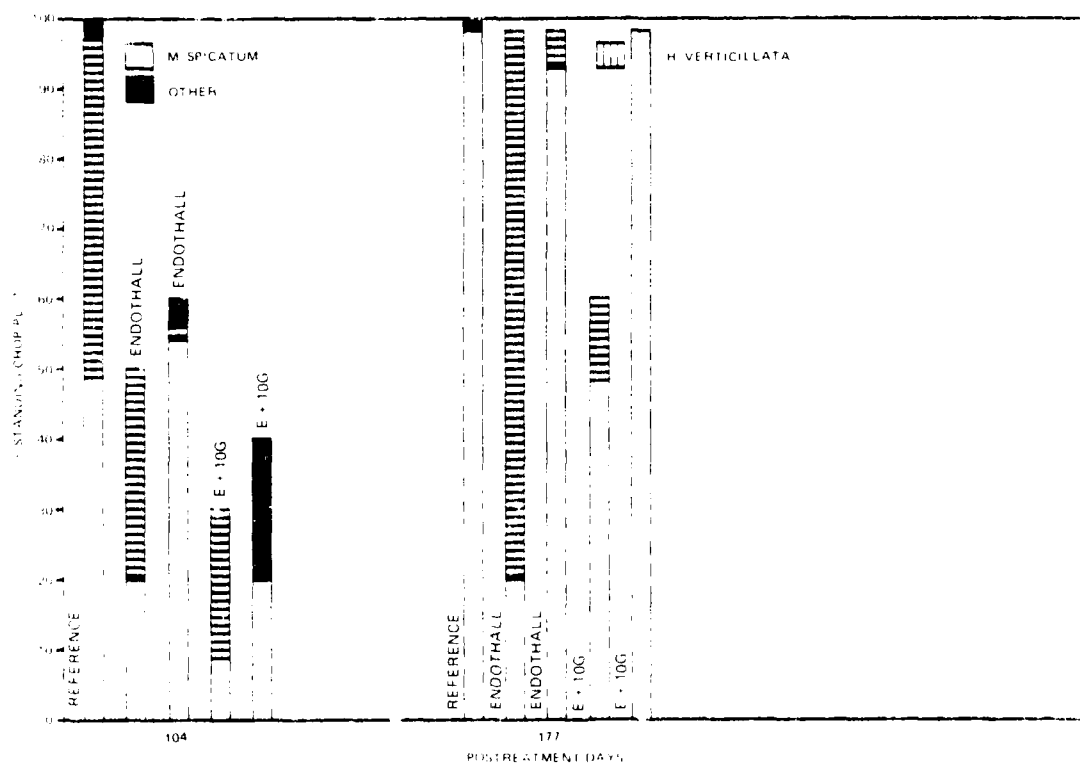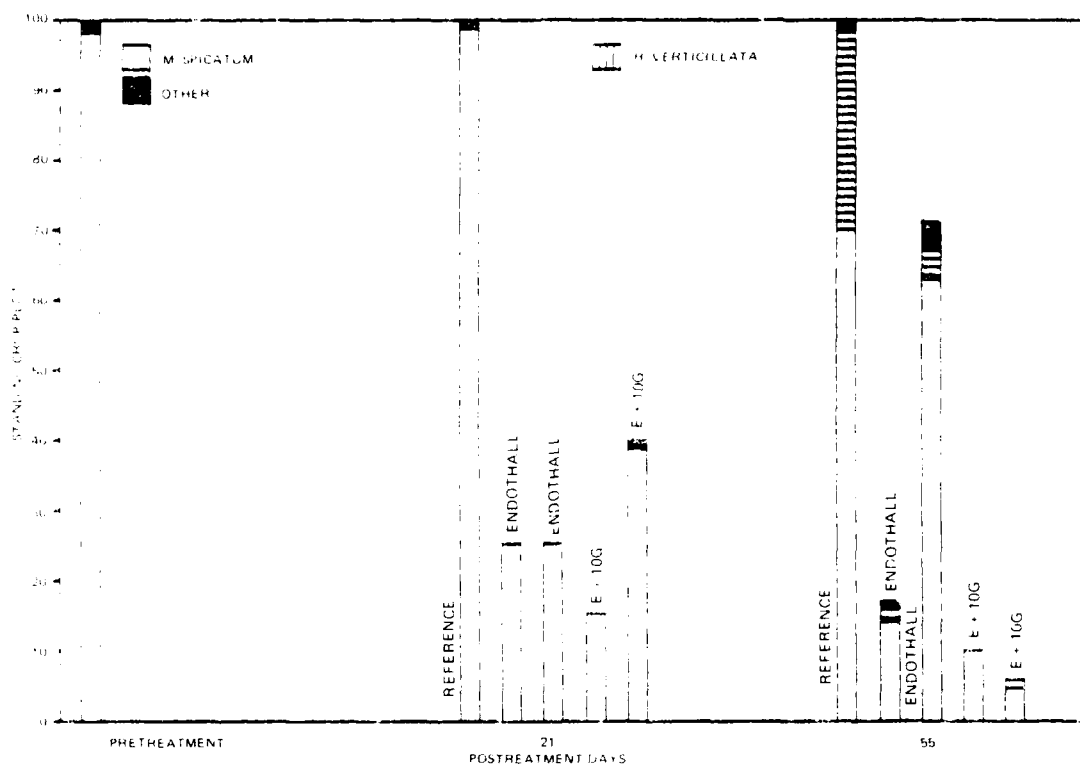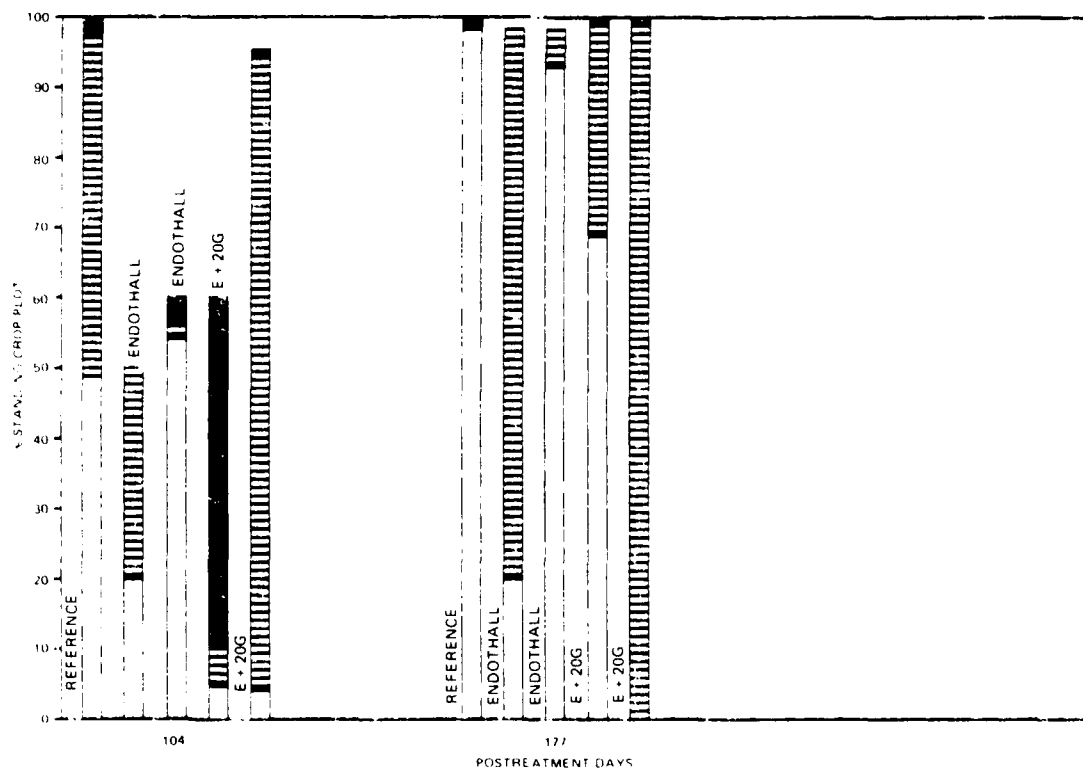
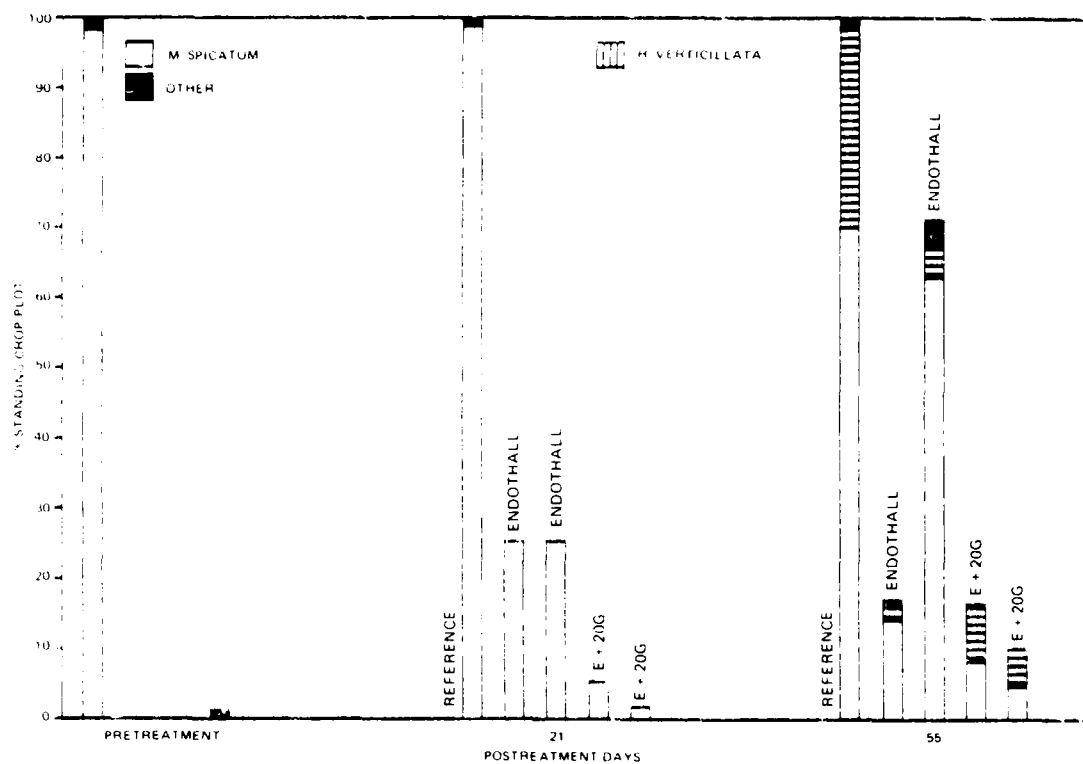Figure 1. Results of dichlobenil 10G efficacy study

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Figure 2. Results of dichlobenil 20G efficacy study
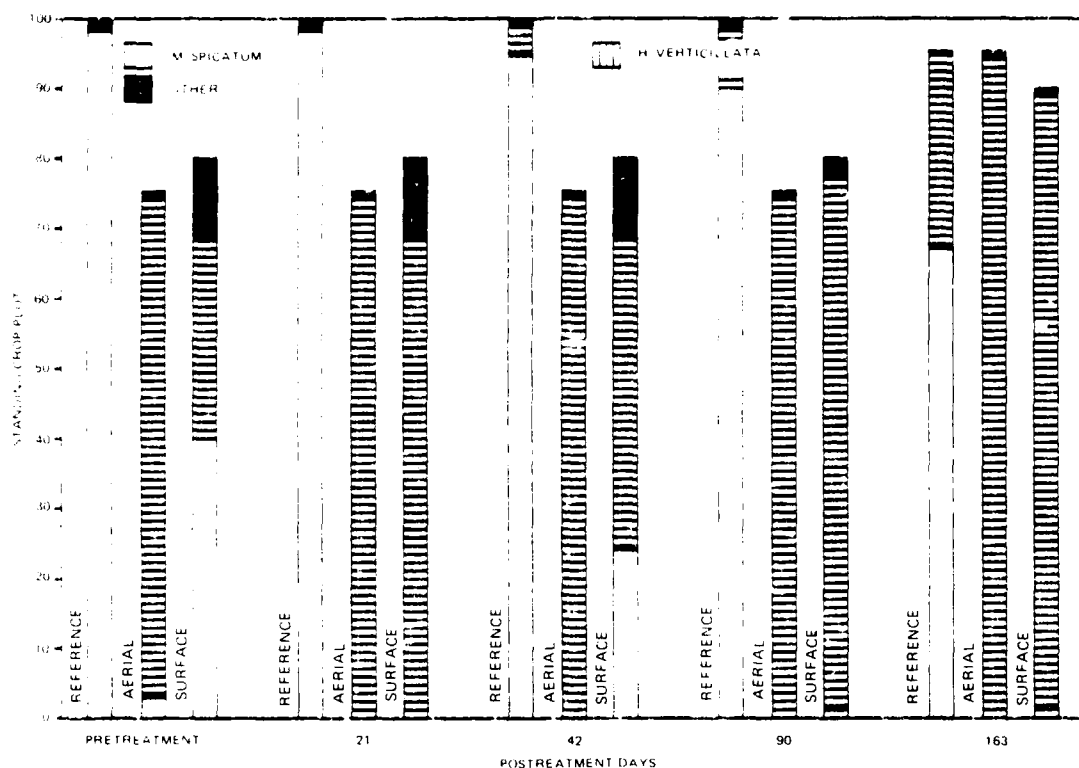
178

Figure 3. Results of triclopyr efficacy study

days 90 and 163. This was a result of an increase in the *H. verticillata* population, not in the *M. spicatum* population.

Triclopyr had a substantial effect on *M. spicatum*. In fact, it reduced the indigenous population to zero within 21 days. Reestablishment of this population was inhibited. This was probably due to the enormous *H. verticillata* population and its competitive advantage associated with the environmental conditions. Even within the reference plot, *H. verticillata* began to increase in abundance toward the end of the evaluation period.

No adverse effects on clams and crawfish, however, were observed during the study period.

CONCLUSIONS

The results of these studies, when analyzed completely, will provide valuable information concerning the dissipation and environmental fate of the chemicals involved. Efficacy evaluations indicate that both dichlobenil and triclopyr are effective in controlling growth of aquatic vegetation. Recommendations for the use of these chemicals are premature until the residue analysis are completed for both studies, including the EUP studies for dichlobenil during FY 87.

Herbicide Adjuvant Evaluation in Flowing Water

by
Kurt D. Getsinger*

INTRODUCTION

One method of managing submersed vegetation in flowing water is to use herbicide/adjuvant mixtures. Adjuvants (e.g., inverting oils and polymers) are designed to increase the effectiveness of liquid herbicide formulations by enhancing the placement of herbicides on target vegetation. Adjuvants allow herbicides to sink and adhere more readily to plant surfaces and, theoretically, increase herbicide contact time. A primary concern is the length of time adjuvants can hold herbicides in the vicinity of target plants when exposed to various flow velocities. Another management approach is to control vegetation with controlled-release (CR) herbicide formulations. These formulations release low levels of herbicides over long periods. The success of both approaches is dependent upon herbicide concentration and exposure time.

The objectives of the herbicide/adjuvant evaluations are to determine which adjuvant or CR formulations show potential in controlling submersed weeds in flowing water and to compare these formulations with conventional herbicides. Phase I of the studies** dealt with the application of various 2,4-D/adjuvant formulations on Eurasian watermilfoil (*Myriophyllum spicatum* L.). Endothall/adjuvant mixtures were utilized in Phase II of the studies, while a CR fluridone formulation was used in Phase III. The preliminary results of Phases II and III, reported here, will be incorporated into Aquatic Plant Control Research Program technical reports. Subsequent studies will be expanded to include additional herbicide/adjuvant combinations and CR formulations.

MATERIALS AND METHODS

Phase II: endothall/adjuvant evaluations

Experiments were conducted in outdoor hydraulic channels (~110 m long × 4 m wide × 2 m deep) located at the Tennessee Valley Authority Aquatic Research Laboratory (TVA/ARL), Browns Ferry, Ala. The channels were modified to contain a series of subchannels (~7 m long × 1 m wide × 1 m deep) which were used for duplicate treatments of each formulation (Figure 1). Apical shoots (~15 cm long) of Eurasian watermilfoil were planted, several centimetres deep, in the
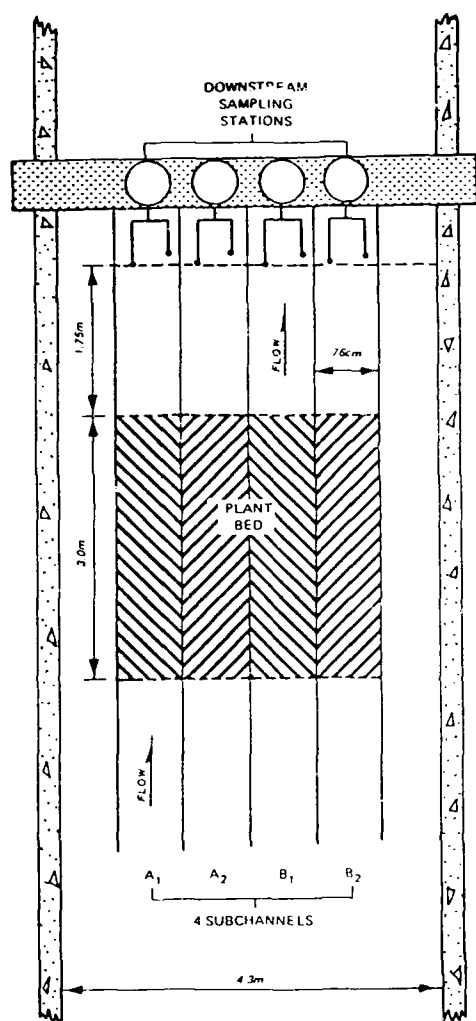

Figure 1. Overhead view of flume channels used in endothall/adjuvant evaluations

bottom of the channels in a mud sediment, capped with 5 cm of washed sand. Shoots were bundled in groups of three and planted 5 to 10 cm apart to produce stands ~3 long × 0.8 m wide, consisting of >1,000 shoots each. Plant stands were allowed to grow for 4 weeks to a height of ~70 cm.

Flow velocities were measured with a Model 201 Marsh-McBirney Flowmeter (accuracy ±2 percent). Water depth was held at 70 cm, and a constant incoming flow velocity was maintained during all experimental runs.

Adjuvants used in this study included the inverting oils Asgrow 403 and I'vod, and the polyments Nalquatic and Poly Control. Aquathol K, a liquid formulation of endothall, was used as the test herbicide. Aquathol K is a contact herbicide that is registered for use in slow-moving water and is effective in controlling Eurasian watermilfoil. Inverting oils were blended with water and endothall to form a thick, mayonnaiselike invert material using a 7:1 water-to-oil ratio. Polymers were blended with water and endothall, using a 2.5-percent polymer, to form a thick, mucouslike material. All herbicide formulations were prepared to provide an endothall treatment rate of 5 mg ai/l. These formulations were transferred to a pressurized spray system and injected below the water surface, throughout the plant stands.

Water samples were collected in the center of each channel, 175 cm downstream from the plant stands, using an ISCO Model 2100 automatic water sampler adapted to sample a water column depth of 10 to 60 cm. Discrete, 100-ml samples were collected every 2 min posttreatment and composited to give a 600-ml sample, representing a 12-min interval. This procedure was continued for 3 hr posttreatment. Water samples were also collected 5 m upstream from each channel every hour during experimental runs to monitor for possible herbicide contamination.

Samples were analyzed for endothall residues by the FDA Analytical Method for Determination of Endothall. Endothall recovery from samples was 84 percent, based on percent recovery of spiked water samples. Endothall analyses were performed by Industrial Laboratories Company, Denver, Colo., under contract to the TVA Laboratory Branch, Chattanooga, Tenn.

Residue data reported in this study represent the mean of two composited samples (one from each channel) for each specific time interval.

Phase III: CR fluridone evaluations

Experiments were conducted in eight outdoor hydraulic channels (previously described) at the TVA/ARL facility. The channels were modified to contain a subchannel as shown in Figure 2. Stands of Eurasian watermilfoil (6 m long × 1.2 m wide) were established from apical cuttings in the subchannels. Plants were allowed to grow for 5 weeks to a height of ~70 cm.

Flow velocities were measured with Model 201 Marsh-McBirney flowmeters (accuracy ±2 percent). Water depth was held at 70 cm, and a constant incoming flow velocity of 1.5 or 3 cm/sec was maintained during all experimental runs.

Two slow-release formulations of fluridone were evaluated in duplicate channels at flow velocities of 1.5 and 3 cm/sec. One of the formulations (CR fluridone) consisted of a polycaprolactone fiber, designed to release fluridone for 21 days following application. The other formulation, a commercial furidone pellet (Sonar 5P), is designed to release fluridone for 7 to 10 days following application. Duplicate channels were treated at labeled rates for each formulation at both flow velocities. Four plant stands were established ~30 m upstream from treated stands and were used as reference plots.

Water samples were collected in the center of and 1.75 m downstream from each plant stand with an ISCO Model 2100 water sampler adapted to sample a water column depth of 10 to 60 cm. Discrete 600-ml water samples were collected posttreatment at 0, 5, 15, and 30 min; 1, 2, 3, 6, and 24 hr; and 3, 7, 14, and 21 days at each sampling location. Samples were analyzed for fluridone residues by high pressure liquid chromatography.

Efficacy was monitored visually and recorded on film at 1, 2, 3, 4, 6, 8, 10, and 12 weeks posttreatment.

Figure 2. Overhead view of flume channel used in fluridone evaluations
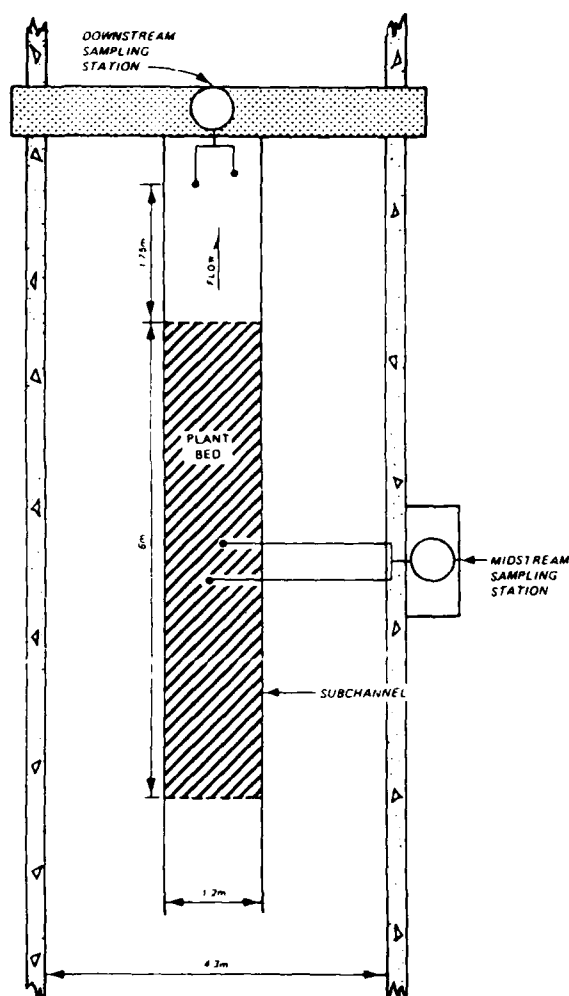
RESULTS

Phase II: endothall/adjuvant evaluations

The inverting oils, Asgrow 403 and I'vod, formed thick, mayonnaiselike mixtures when blended with water and endothall at recommended proportions. Following application, the invert formulations adhered readily to the leaves and stems of the target plants. The amount of invert "flakes" that floated to the surface was minimal due to the subsurface application technique. Invert "flakes" were observed on the plants up to 30 hr posttreatment.

The thick, mucouslike polymer formulations sank rapidly and adhered to the target plants in the form of long strings or large droplets. Remnants of the polymer formulation were visible on the plants for only 4 to 6 hr posttreatment.

Endothall residues from all of the formulations tested were measurable above the 0.01 mg/l detection limit, up to 84 min posttreatment when herbicide mixtures were applied at flow velocities of 1.5 cm/sec. Endothall concentrations fell below detection, however, by 24 min posttreatment when using the conventional, liquid formulation of Aquathol K (Figure 3). The use of invert formulations results in
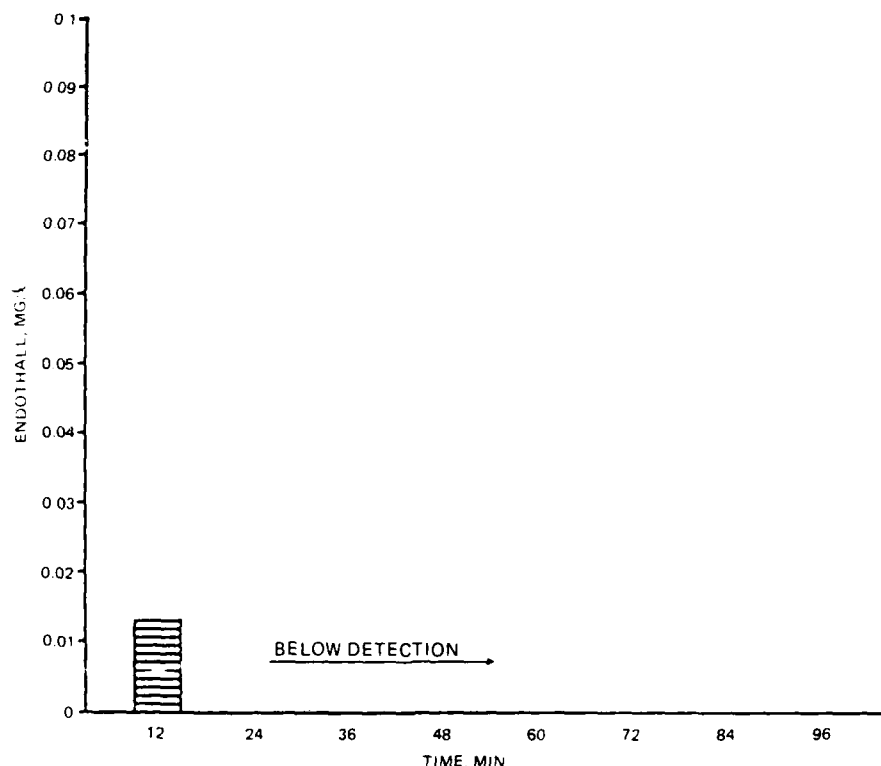
Figure 3. Effect of time on endothall residues 1.75 m down-
stream of plant stands using a conventional liquid formulation
of Aquathol K at a flow velocity of 1.5 cm/sec

endothall release profiles of 36 min posttreatment with Asgrow 403 and 48 min posttreatment with I'vod (Figures 4 and 5). The highest endothall concentration (0.045 mg/l at 24 min posttreatment) was measured using the invert formulation Asgrow 403.

The longest herbicide release profiles found in this study were achieved with the polymer formulations (Figures 6 and 7), as endothall concentrations fell below detection at 72 min posttreatment with Nalquatic and at 84 min posttreatment with Poly Control. The highest endothall concentration measured using the polymer formulations was 0.035 mg/l, with Nalquatic at 24 min posttreatment.

In contrast, endothall residues from all of the formulations tested were below detection at 12 min posttreatment when herbicide mixtures were applied at flow velocities of 3 cm/sec.

Since the experiments were terminated at 3 hr posttreatment, plant efficacy was not evaluated. Water samples collected 5 m upstream from each plant stand during experimental runs showed no herbicide contamination.

Phase III: CR fluridone evaluations

Only preliminary results are available at this time. Efficacy evaluations indicated that the target plants were not controlled by any of the formulations through 12 weeks posttreatment. Fluridone residues are in the process of being analyzed.
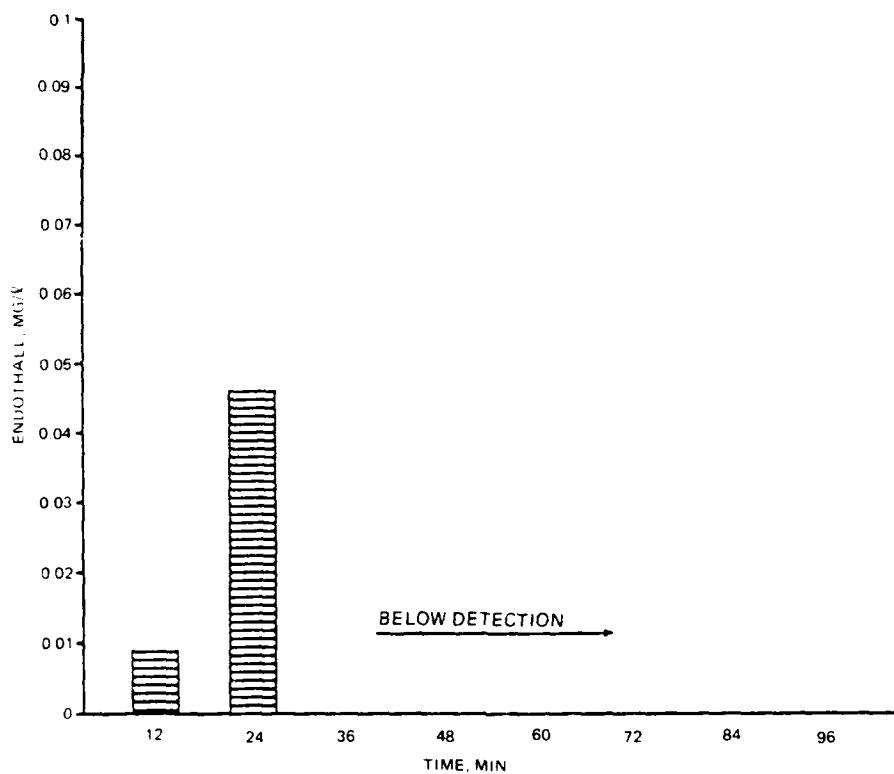
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Figure 4. Effect of time on endothall residues 1.75 m down-
stream of plant stands using a formulation of Asgrow 403/
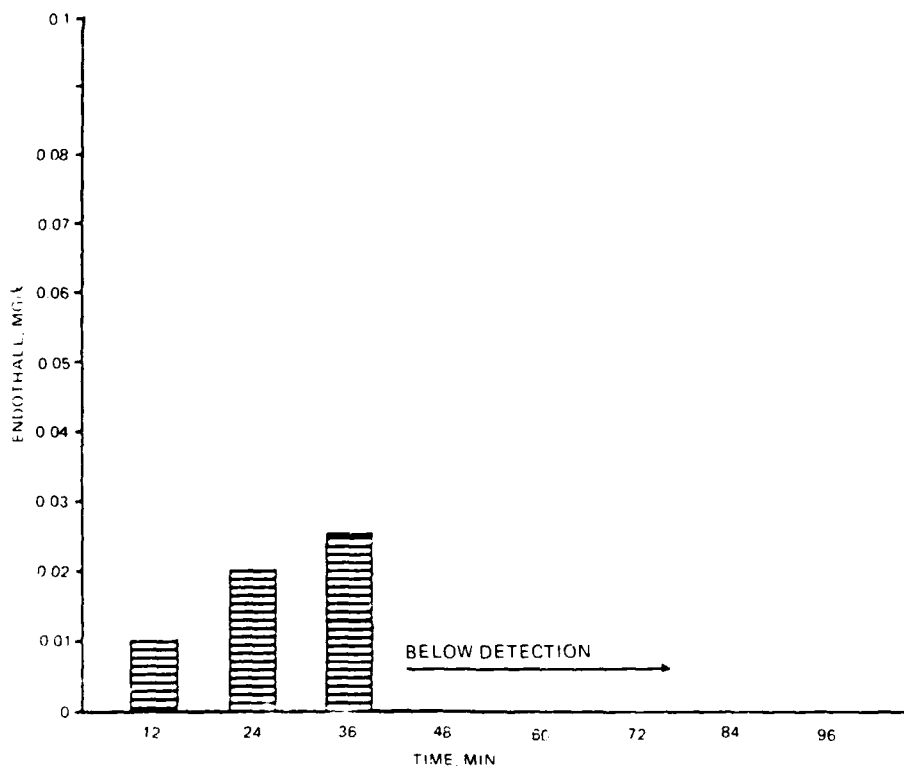endothall at a flow velocity of 1.5 cm/sec

Figure 5. Effect of time on endothall residues 1.75 m down-
stream of plant stands using a formulation of I'vod/endothall
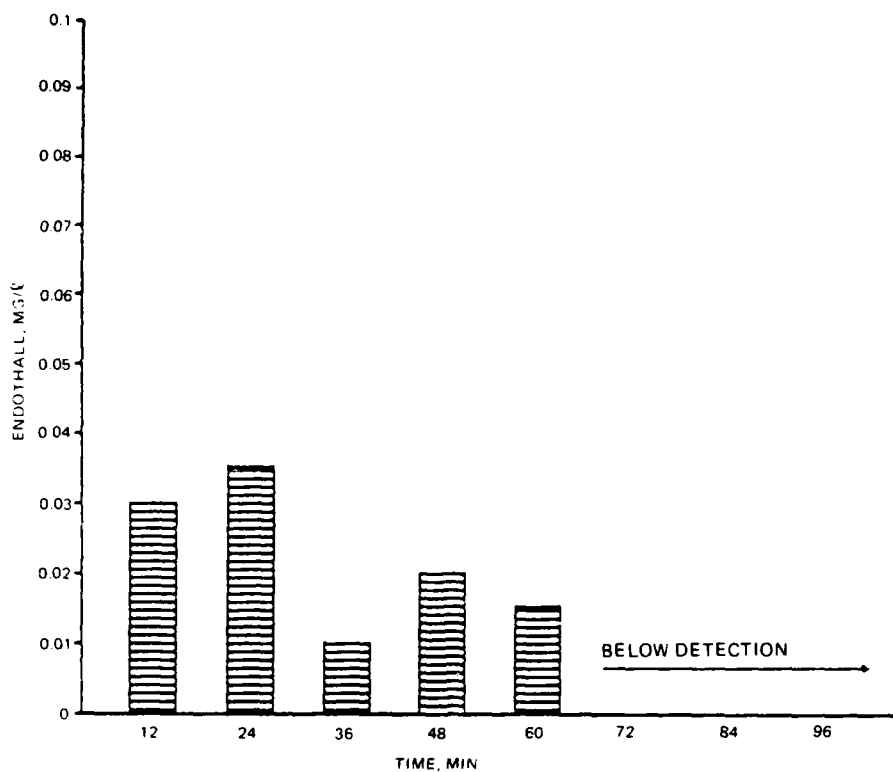at a flow velocity of 1.5 cm/sec

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Figure 6. Effect of time on endothall residues 1.75 m down-
stream of plant stands using a formulation of Nalquatic/
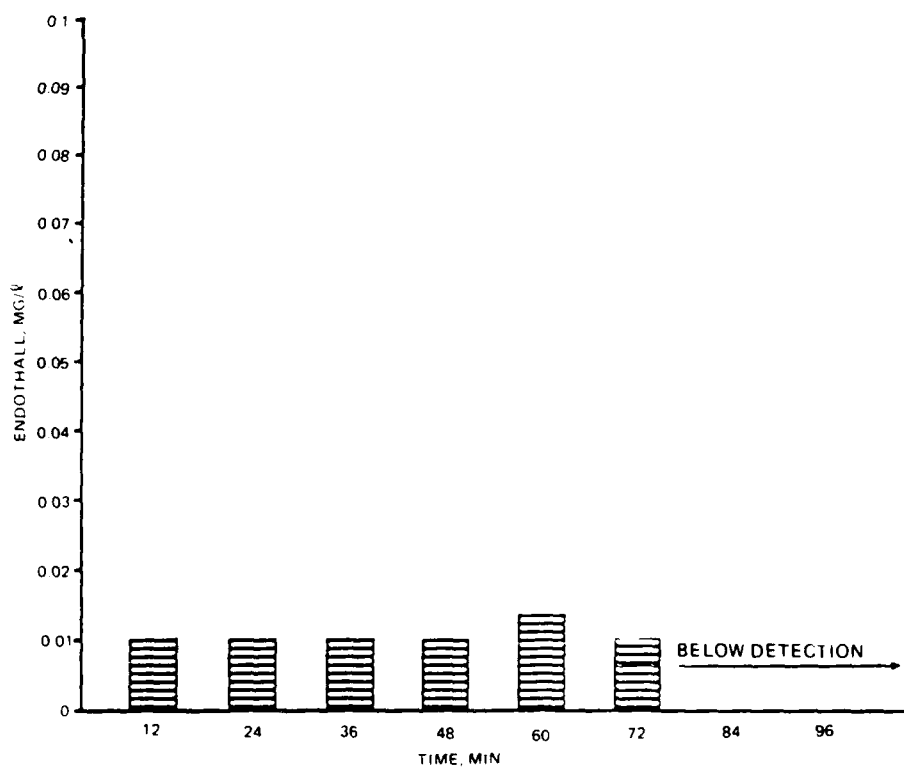endothall at a flow velocity of 1.5 cm/sec

Figure 7. Effect of time on endothall residues 1.75 m down-
stream of plant stands using a formulation of Poly Control/
endothall at a flow velocity of 1.5 cm/sec

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CONCLUSIONS

Based on results from the endothall/adjuvant evaluations, Nalquatic and Poly Control show the greatest potential as effective adjuvants for the release of endothall in flowing water, where flow velocities within plant stands are <1.5 cm/sec. However, none of the adjuvant formulations tested seem to have an advantage over the conventional, liquid endothall formulation when applied to plant stands where water velocities within the stands are >3 cm/sec. Also, results from this study and results from a previous 2,4-D/adjuvant study* suggest that hydrophilicity of a herbicide plays a role in determining herbicide release profiles from adjuvant mixtures. A hydrophilic herbicide (e.g., endothall) is released more rapidly from adjuvants than a hydrophobic herbicide (e.g., 2,4-D).

Preliminary results of the fluridone evaluation indicate that neither CR fluridone nor Sonar 5P controlled Eurasian watermilfoil under the test conditions. The use of CR and granular formulations of fluridone in flowing water (in the field and in experimental channels) has resulted in poor control of submersed plants. Field-type studies, designed to determine the concentration/exposure time requirements for controlling plants in flowing water, are needed to resolve this problem.

FUTURE RESEARCH

Studies in FY 87 will evaluate liquid fluridone formulations for the control of submersed plants in flowing water. These studies will be the first phase in an effort to determine concentration/exposure time relationships under field conditions. The studies will be conducted in the TVA channels at Browns Ferry in cooperation with Elanco Products Company and TVA.

ACKNOWLEDGMENTS

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* US Food and Drug Administration. 1973. "Analytical Method for Determination of Endothall. No 180.293." Pesticide Analytical Manual II.

Herbicide Application Technique Development
for Flowing Water

by
Kurt D. Getsinger*

INTRODUCTION

Recent studies suggest that herbicide concentration/exposure time is a critical factor for successfully controlling submersed plants.**[†,‡] Maintaining an optimum herbicide concentration and exposure time in flowing-water environments has proven to be extremely difficult. Few, if any, reliable herbicide application techniques are available to achieve the necessary concentration/exposure time relationships required for controlling submersed plants in these environments. Flowing water directly affects the concentration/exposure time relationship; therefore, a better understanding of flow velocities within submersed plant stands is needed before application techniques can be identified or developed.

The objectives of this study are to characterize flow velocities in submersed plant stands under field conditions, and to identify and/or develop application techniques to maximize herbicide contact time and efficacy in flowing-water environments. Field locations utilized in this study were streams, rivers, irrigation/drainage canals, and estaurine tidal areas.

The preliminary results reported are derived from a larger investigation, the results of which will be reported in an Aquatic Plant Control Research Program technical report.

MATERIALS AND METHODS

Flow velocities were measured in stands of Eurasian watermilfoil (*Myriophyllum spicatum* L.), and hydrilla (*Hydrilla verticillata* Royle), grown in hydraulic flumes at the Waterways Experiment Station (WES) and the Tennessee

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* US Army Engineer Waterways Experiment Station, Vicksburg, Mississippi.

** H. E. Westerdahl and J. F. Hall. 1983. "The 2,4-D Threshold Concentrations to Control Eurasian Watermilfoil and Sago Pondweed," *Journal of the Aquatic Plant Management Society*, Vol 21, pp 22-25.

† J. F. Hall. 1985. "Determination of Fluridone Concentration/Exposure Time Relationship for the Control of *Myriophyllum spicatum* and *Hydrilla verticillata*," *Proceedings, 19th Annual Meeting, Aquatic Plant Control Research Program*, Miscellaneous Paper A-85-4, US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

‡ T. K. Van and R. D. Conant, Jr. "Chemical Control of Hydrilla in Flowing Water: Herbicide Uptake Characteristics and Concentrations versus Exposure," Technical Report in preparation, US Army Engineer Waterways Experiment Station, Vicksburg, Miss.

Valley Authority-Aquatic Research Laboratory (TVA-ARL), at Browns Ferry, Ala.) using Model 201 Marsh-McBirney flowmeters (accuracy ±2 percent). Flows were also measured in stands of wild celery (*Vallisneria americana* Michaux.), sago pondweed (*Potamogeton pectinatus* L.), and water stargrass (*Heteranthera dubia* (Jacquin) MacM.) in the Holston River near Kingsport, Tenn., using a Model PVM-2A Montedoro-Whitney portable velocity monitor (accuracy ±2 percent).

Plant stands in the hydraulic flumes were relatively small (3 to 6 m long, 0.8 to 1.2 m wide, and 0.7 m deep) and incoming flow velocities ranged from 1.5 cm/sec (0.05 ft/sec) to 18 cm/sec (0.6 ft/sec). Plant stands in the Holston River were 0.4 to 1.2 ha (1 to 3 acres) in size and 0.75 to 1 m deep. Incoming flow velocities were 20 to 25 cm/sec (0.67 to 0.84 ft/sec).

RESULTS

Flow velocities were considerably less inside submersed plant stands, compared with outside the stands. This condition existed in the experimental hydraulic flumes as well as in the field. Table 1 shows flow velocities in a flume planted with Eurasian watermilfoil. As flow velocities upstream from the plant stands increased (18 cm/sec), the stands began to bend, with the flow, into a horizontal position. Flow velocities in the water column above the stands accelerated (33 cm/sec), yet velocities within the stands remained low (4.5 cm/sec).

Flow velocities within field stands (ie., Holston River) followed patterns measured in the experimental flumes. Figure 1 depicts a typical flow velocity pattern, in a wild celery stand in the Holston River. Incoming velocities were noticeably dampened 0.5 m inside the stand and dramatically reduced at the 2-m mark. The greatest velocity reduction occurred in the bottom half of the stand. The center channel incoming velocity of ~20 cm/sec (0.67 ft/sec) was reduced to <4 cm/sec (0.13 ft/sec) in 90 percent of the stand's vertical profile, and to <0.1 cm/sec (0.003 ft/sec) in 50 percent of the profile.

Table 1
Flow Velocities Measured in Flume System Containing Plant Stands

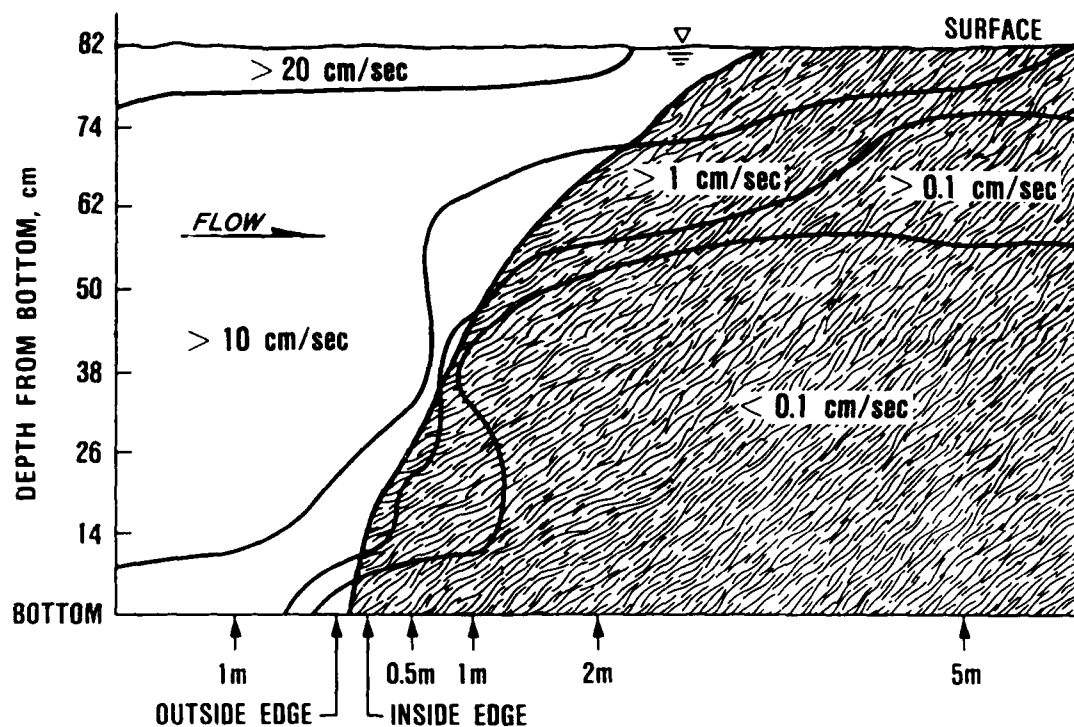|	Flow Velocity, cm/sec		
Upstream Edge of Plant Stand	Within Plant Stand	Above Plant Stand	Downstream Edge of Plant Stand
1.5 (0.05)*	1.5	1.5	1.5
3.0 (0.1)	3.0	3.0	3.0
6.0 (0.2)	3.9	11.7	6.0
12.0 (0.4)	3.9	27.0	13.5
18.0 (0.6)	4.5	33.0	16.6

*Feet per second.

Figure 1. Flow velocity in a submersed macrophyte stand

CONCLUSIONS

Flow velocities are greatly reduced upon entering submersed plant stands, particularly toward the bottom. Therefore, herbicide placement and delivery techniques will be critical factors in the successful control of submersed plants in flowing-water environments. An understanding of flow velocities within submersed plant stands is a necessary element in the identification and development of herbicide application techniques in flowing water.

PRESENT AND FUTURE RESEARCH

A pilot study was initiated in the WES hydraulic flume to evaluate 2,4-D release profiles from granular formulations applied with different techniques. An even-distribution, broadcast technique was compared with a perforated-pipe, suspension technique. Efficacy and water residue data are presently being analyzed. Flow velocity studies will continue at various field sites throughout the country, and a field study to evaluate herbicide application techniques in tidal areas will be initiated in Florida during FY 87.

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Plant Growth Regulators for Aquatic Plant Control

by
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INTRODUCTION

Although aquatic weeds can have detrimental effects on a body of water, some aquatic plant growth is considered desirable. For example, submersed aquatic plants provide oxygen through photosynthesis, habitat for fish and fish food organisms, and bottom sediment stabilization. Unfortunately aquatic weed management is usally all-or-nothing, since the primary tool is the use of aquatic herbicides, most of which are nonselective and kill all of the plants in the area of treatment. Rapid plant decomposition may result in adverse effects on the other components of the aquatic system.

Another potential approach to managing aquatic vegetation is to use plant growth regulators (PGRs). Many PGRs are synthetic versions of plant hormones. Some are used as herbicides (e.g., 2,4-D); others are used in crop production systems to increase (or decrease) growth rates, enhance (or slow) flowering or ripening, or modify properties such as color or texture (Nickell 1982). Some PGRs are antihormones that either prevent the expression of the naturally occurring hormone or inhibit its synthesis.

One group of compounds currently in commercial development is the antigibberellins (Lever, Shearing, and Batch 1982; Rademacher et al. 1984). As naturally occurring plant hormones, gibberellins are primarily responsible for stem elongation. Several antigibberellins have been found to reduce stem length of terrestrial plants in species ranging from grasses to trees without altering viability or morphological differentiation (e.g., seedhead development) (Lever, Shearing, and Batch 1982; Kaufmann 1985). At least one of the antigibberellins, paclobutrazol, was found to inhibit a series of oxidative steps specific to the biosynthesis of gibberellin (Hedden and Graebe 1985).

The principal objective of this Corps of Engineers-supported project is to determine if antigibberellin-type PGRs can reduce the rate of stem elongation in submersed aquatic plants without killing the plants. This would lead to a lawn or "turf" at the bottom of the body of water that would not be weedy because the plants are short, but would be composed of functional plants able to provide oxygen, habitat and bottom stabilization.

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The general design of the project is to use a bioassay procedure to evaluate antigibberellin activity on *M. spicatum* (Eurasian watermilfoil) and *H. verticillata*. The specific objectives are to determine:

- The effects of PGRs on stem length and other associated length and biomass parameters (growth responses).
- The effects of PGRs on the physiological competency of the plants, with emphasis on oxygen production (physiological responses).
- Length of time in which PGR effects persist.
- Effective exposure times for PGR effects to be expressed.

The antigibberellins to be tested are flurprimidol (Eli Lilly and Company), paclobutrazol (ICI Americas), and uniconazol (Chevron Chemical Company and Sumitomo). These compounds are applied to either soil or foliage in terrestrial systems and show a half-life of less than 6 months in soil. Table 1 summarizes the environmental data available on these three compounds.

This paper discusses the first 3 months of the project, the goals of which were to establish cultures of *M. spicatum* and *H. verticillata* and to begin to develop an effective concentration range for one PGR.

Table 1
Properties of Three Triazole Derivatives Used as Antigibberellins*

Parameter	Flurprimidol	Paclobutrazol	Uniconazol
Use rate in terrestrial systems, lb ai/acre (ppm**)	0.5-2 (0.5-)	0.5-1 (0.5-1)	0.03-0.06 (0.03-0.06)
Water solubility, ppm	120-140	35	14.3
Toxicity			
Rainbow trout			
96 hr LC_{50}	18.3 ppm	33.1 ppm	No data
No observed-effect level	0.5	10.0 ppm	No data
Carp, 49 hr LC_{50}	No data	No data	6.4 ppm
Subchronic toxicity			
Rats, no observed-effect level	25 ppm	No data	No data
Mutagenicity	Negative	No data	Negative

* Data from company technical bulletins and reports.

** Approximate (calculated on basis of compound sprayed on the soil surface and incorporated to a depth of 8 cm).

MATERIALS AND METHODS

Algal-free cultures of *M. spicatum* and *H. verticillata* were obtained from Dr. John Andrews of the University of Wisconsin and Dr. Steve Klaine of Memphis State University, respectively. Hydrilla was grown in 10-percent Hoagland's solution and watermilfoil in a modified Gerloff's solution (Andrews 1980). Stock cultures of both plants were maintained in controlled environment chambers at 25° C, 200 $\mu E/m^2/sec$, and a 16:8 light/dark photoperiod. Watermilfoil cultures were bubbled continuously with CO_2-enriched air.

Four-centimeter-long lateral shoot segments were excised from parent plants and transferred to 250-ml flasks (one shoot per flask) with 150-ml culture medium and the appropriate amount of PGR. The PGR tested was paclobutrazol* (PP333) at concentrations of 0.015 to 3.0 mg ai (active ingredient)/l.

The experimental flasks were placed under the same growing conditions as stock cultures but were not provided with CO_2. The data shown are results of watermilfoil culture after 28 days exposure and hydrilla after 14 days exposure.

RESULTS

Elongation of the primary stem segment of watermilfoil was inhibited at all paclobutrazol concentrations (Table 2). Stem length at the lowest paclobutrazol concentration (0.015 mg ai/l) was 46.5 percent that of the untreated. Maximum inhibition of stem elongation (lengths approximately 18 percent of the untreated) was observed at the three highest concentrations used. These plants did not increase in length over the 28-day exposure period, whereas untreated plants increased in length 3.6 times over the original 4-cm segment. Plants at the three highest concentrations were brittle and tended to fall apart easily.

Table 2

Effect of Paclobutrazol on Main Stem Length
in *Myriophyllum spicatum* after 28 Days Exposure

Paclobutrazol mg ai/l	Main Stem Length* cm	Percent of Untreated	Times Increase over Initial
0.0	14.4 a	—	3.6
0.015	6.7 b	46.5	1.7
0.15	4.7 c	32.6	1.2
0.75	4.2 c	29.2	1.1
1.5	4.1 c	28.5	1.0
3.0	4.0 c	27.8	1.0

* Values followed by the same letter are not significantly different at the 5-percent level as determined by the Student-Neuman-Keuls Test. Each value is the mean of three replicates.

Hydrilla shoot growth was also inhibited at all paclobutrazol concentrations tested (Table 3). After 14 days of exposure, stem lengths of treated plants ranged from 55 to 77 percent of untreated stem lengths (Figure 1). Unlike watermilfoil, all treated plants grew during the test period, exhibiting increases of 2 to 2.9 times the initial stem lengths. In an earlier preliminary study, exposure of hydrilla segments to 3.5 mg ai/l for 14 days resulted in stem length inhibition of 42 percent in comparison to untreated plants and no increase in length over the initial 4 cm. However, these segments were red in color, brittle, and not very healthy in appearance.

* (2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-1,2,4-triazol-1-yl)pentan-3-ol.

Table 3

Effect of Paclobutrazol on Main Stem Length
in *Hydrilla verticillata* after 14 Days Exposure

Paclobutrazol mg ai/ℓ	Main Stem Length* cm	Percent of Untreated	Times Increase over Initial
0.0	14.9 a	—	3.7
0.015	10.8 b	72.5	2.7
0.15	11.5 b	77.2	2.9
0.75	10.9 b	73.2	2.7
1.5	9.9 bc	66.4	2.5
3.0	8.2 c	55.0	2.0

* Values followed by the same letter are not significantly different at the 5-percent level as determined by the Student-Neuman-Keuls Test. Each value is the mean of three replicates.

Figure 1. Untreated hydrilla (left) and hydrilla exposed to 0.75 mg ai/ℓ paclobutrazol for 14 days (right). Original shoot length: 4 cm. (These plants are from experiments different from those described in this paper.)

Data on lateral stem lengths suggest that low paclobutrazol concentrations may have a slight stimulatory effect on overall growth of hydrilla rather than an inhibitory one (Table 4). The number of lateral shoots produced at 0.015 and 0.15 mg ai/l was higher than the number in the untreated controls. At 0.015 mg ai/l, the additional lateral stem growth plus main stem growth resulted in plants that were, in overall length and increase over initial lengths, slightly (although not significantly) greater than the untreated plants. This was the case even though lengths per branch were shorter than lengths per branch in the untreated plants. No lateral shoots were produced at 0.75 mg ai/l or above.

An apparent proliferation of roots was also observed at the two low concentrations (Table 5). Even though length per root was less, total root length at these two concentrations was as high or higher than in untreated plants. Paclobutrazol at 0.75 mg ai/l resulted in very short root lengths.

Table 4
Effect of Paclobutrazol on Lateral Stems
and Total Lengths in *Hydrilla verticillata* after 14 Days Exposure

| Paclobutrazol mg ai l | No. | Lateral Stems | | Laterals + Main Stem | | |
		Total Length* cm	Length/ Lateral* cm	Total Length* cm	Percent of Untreated	Times Increase Over Initial
0.0	1.3 a	6.1 a	4.7 a	21.0 a	—	5.3
0.015	4.0 b	11.0 b	2.8 b	21.8 b	103.8	5.5
0.15	2.7 c	7.1 a	2.6 b	18.6 b	88.6	4.7
0.75	0 d	—	—	10.9 a	51.9	2.7
1.5	0 d	—	—	9.9 cd	47.1	2.5
3.0	0 d	—	—	8.2 d	39.0	2.0

* Values followed by the same letter are not significantly different at the 5-percent level as determined by the Student-Neuman-Keuls Test. Each value is the mean of three replicates.

Table 5
Effect of Paclobutrazol on Root Lengths
in *Hydrilla verticillata* after 14 Days Exposure

Paclobutrazol mg ai/l	No. Roots*	Total Root Length* cm	Length/ Root* cm
0.0	4.7 b	62.6 b	13.3 a
0.015	9.3 a	83.6 a	9.0 b
0.15	7.7 a	67.3 ab	8.7 b
0.75	2.0 bc	5.3 c	2.7 c
1.5	1.3 c	3.1 c	2.4 c
3.0	1.4 c	1.7 c	1.3 c

* Values followed by the same letter are not significantly different at the 5-percent level as determined by the Student-Neuman-Keuls Test. Each value is the mean of three replicates.

DISCUSSION AND FUTURE RESEARCH

These results, although preliminary, suggest that paclobutrazol has an inhibitory effect on stem elongation in submersed aquatic plants. The plants (with the exception of the highest concentrations) look "normal," although viability cannot be established until we begin taking oxygen evolution measurements.

Hydrilla appears to be more tolerant to paclobutrazol than watermilfoil. Hydrilla plants at all concentrations had grown in relation to their initial lengths, whereas the watermilfoil plants did not exhibit any elongation at the three highest paclobutrazol concentrations, although they had an additional 14 days to grow. The production of lateral shoot and root primordia may be stimulated at lower concentrations of the PGR, but the antigibberellin does appear to retard their elongation.

Studies are not continuing to narrow the effective concentration range (concentrations betweeen those which are toxic and those which are stimulatory to growth) for both plants, with emphasis being placed on the 0.75 to 3.0 mg/l concentrations for hydrilla and concentrations less than 0.75 mg/l for watermilfoil.

Next year we plan to obtain a complete set of data on the effects of at least one, and perhaps two, of the PGRs on both growth and physisological parameters of the two test species.

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ECOLOGY STUDIES

Ecology of Submersed Macrophytes
an Overview

by
John W. Barko*

INTRODUCTION

Past studies conducted by this program and focused on increasing current understanding of the ecology of submersed aquatic macrophytes have involved predominantly independent evaluations of effects of environmental factors on macrophyte growth and distribution (Barko 1986). Most recently we have begun to examine these factors in an interactive context, with increasing emphasis on field research to corroborate laboratory study findings. Investigations of the influence of sediment and water chemistry on macrophyte growth continue in order to better elucidate casual mechanisms operative in directing short- and long-term succession. These studies are intended to provide improved information on environmental conditions conducive to the proliferation of weedy species, and are expected ultimately to provide a basis for the development of innovative aquatic plant management techniques.

Although a significant body of knowledge presently addresses the role of the environment in affecting growth of submersed macrophytes, relatively little information is available on the influence of macrophytes themselves on the environment. Submersed aquatic vegetation has long been recognized as being somehow important to ecosystem diversity and productivity. However, quantitative data on specific mechanisms of macrophyte influence on the environment are lacking. In an attempt to improve understanding of macrophyte/habitat relationships, we have recently initiated studies to examine the influences of submersed macrophytes on sediment composition and water chemistry. This work is being conducted in close association with studies addressing variations in the abundance and distribution of invertebrate organisms and fish affected by aquatic vegetation.

Overall objectives of research within the ecology area of the Aquatic Plant Control Research Program are to:

a. Determine the influence of the environment on plant growth and distribution: macrophyte response to environment.

b. Determine the influence of plants on the environment: habitat response to macrophytes.

c. Develop innovative and environmentally compatible plant management approaches based on improved understanding of macrophyte-environment interactions: plant management within an ecological context.

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This article provides an overview of ongoing research and highlights recent findings in view of these objectives. Details of research are contained in referenced articles.

RESEARCH ELEMENTS

Macrophyte response to environment

A variety of environmental factors are known to be important in affecting the productivity, distribution, and species composition of submersed macrophyte communities. Foremost among these are light, water temperature, nutrients (including inorganic carbon), and sediment composition (Barko, Adams, and Clesceri 1986). Light and temperature are key variables driving photosynthesis and reproduction. These variables affect macrophyte morphology and distribution, thereby influencing species composition as well. Sediment properties affect nutrition, and influence macrophyte production. Given broad variations among species in nutrient uptake and retention efficiencies, sediment properties also affect species succession. The availability of dissolved inorganic carbon can be a very important factor influencing the growth potential of a variety of submersed macrophytes, particularly in situations where other essential nutrients are in abundance. Water chemistry parameters have also been correlated with the distribution of submersed macrophyte species.

In articles provided in this report, we examine in detail results of recent investigations designed to evaluate differential responses of monoecious and dioecious *Hydrilla* to a variety of environmental conditions. These comparisons are topical, given the typically adventive nature of the genus *Hydrilla*, and the absence of information on variations in response to environment by different biotypes (US Army Engineer Waterways Experiment Station (USAEWES 1985)). In a study designed to assess response of monoecious *Hydrilla* (among other species) to different levels of salinity, we found that the former was much less tolerant to salinity above about 4 ppt (Twilley et al. 1987) than earlier anticipated (USAEWES 1985). We have also compared the response of both monoecious and dioecious *Hydrilla* to different temperatures on nutritionally favorable (fine-textured, inorganic) and unfavorable (organic) sediments (McFarland and Barko 1987). Growth (biomass yield) and stature (shoot length and number) of both of these biotypes were severely diminished on organic sediment in contrast with growth on inorganic sediment. Both biotypes were critically sensitive to water temperature, with maximum production of biomass occurring between 28° and 32° C. Overall, the monoecious biotype appears better adapted than the dioecious biotype to intermediate thermal conditions. Monoecious *Hydrilla* produced more shoots and a greater number of tubers than dioecious *Hydrilla* under all experimental conditions.

In a series of investigations designed to assess the influence of inorganic carbon availability on the growth of submersed aquatic macrophytes (Smart and Barko 1987), we demonstrated that in eutrophic systems macrophyte growth is potentially limited by carbon supply. Competition among different macrophyte species under eutrophic conditions is probably affected dramatically by variations among species

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in their specific abilities to access inorganic carbon. Sediment nitrogen supply has a direct influence on the growth of submersed macrophytes, and may thereby determine inorganic carbon requirements. In the laboratory, we have been able to induce acute carbon limitation in submersed macrophytes by fertilizing sediment with nitrogen. In nature, variations in the availability of nitrogen, and perhaps other sediment-derived nutrients, are likely to have a significant influence on the carbon economy of submersed macrophyte populations.

Influence of macrophytes on aquatic habitat

Through processes of growth, senescence, and decomposition, aquatic macro phytes can exert a significant influence on the surrounding environment. From a physical/chemical perspective, macrophyte populations potentially influence water and sediment chemistry and hydraulic conditions. Aquatic macrophytes also create structural complexity by providing refuge, nutrition, and substrates for aquatic animals. In a cooperative effort within the Environmental Laboratory, we have initiated detailed examination of the processes and interactions involving environment and submersed aquatic macrophytes (depicted in Figure 1).

During the summer of 1986, we conducted habitat-related studies in Eau Galle Reservoir, Wisconsin, and in the Potomac River, Washington, DC. Our objective was to assess the influence of submersed macrophytes on sediment and water chemistries as they relate to habitat conditions for associated animals (invertebrate organisms and fish). Results of biological studies are detailed in the articles of Miller, Beckett, and Blancher (1987), and Killgore, Morgan, and Hurley (1987).
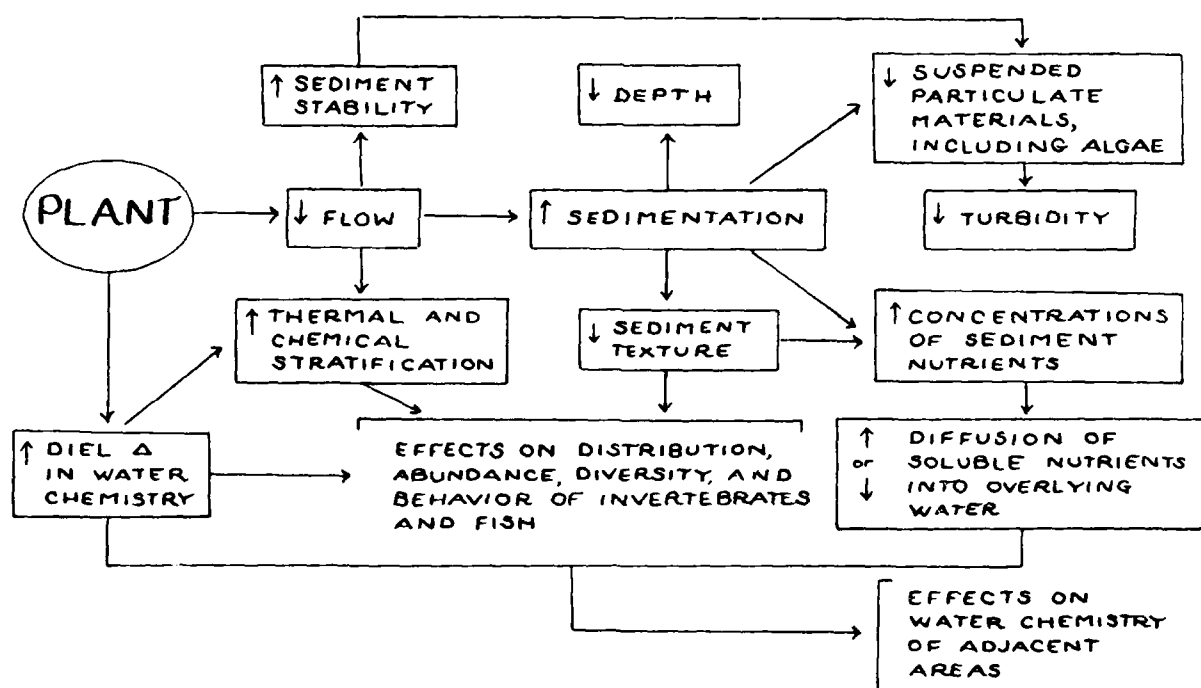

Figure 1. Conceptual model of processes and interactions involving submersed macrophytes and their environment

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In general, we found greater animal population densities and greater biological productivity overall in vegetated than in unvegetated areas in both reservoir and riverine environments.

Because of the intense metabolic activity of submersed macrophyte populations, diel fluctuations in water chemistry were considerably greater in vegetated than in adjacent open water areas (Godshalk, Barko, and James 1987 and unpublished). In the Potomac River, within a near-monotypic stand of *Hydrilla*, we observed variations in pH (7-10) from night to day, equivalent to a three order of magnitude change in hydrogen ion activity. In Eau Galle as well as in the Potomac River, oxygen varied between 10-percent saturation and greater than 200-percent saturation on a daily basis during midsummer. From these results it is apparent that the littoral regions of these systems provide a chemically diverse habitat for occupancy by ani nals.

In our greenhouse facility and in Eau Galle reservoir we examined changes in the vertical distribution of sediment redox potential and nutrient availability during the growth of aquatic macrophytes (Chen, Barko, and Twilley 1987). Plant species with extensive root systems markedly affected redox, resulting in changes from reduced to oxidized conditions in about 6 weeks. Species with poorly developed root systems (e.g., *Hydrilla*) did not alter sediment redox significantly. Altered redox, in combination with nutrient uptake by macrophytes, substantially affected concentrations of essential nutrients in sediments. Changes in sediment chemistry induced by aquatic macrophytes, in addition to influencing subsequent nutrient availability to rooted vegetation, also affect element exchanges with overlying water.

Management within an ecological context

Aquatic systems, even within localized geologic/geographic settings, do not experience 'weed' problems to the same extent; in many cases 'weediness' seems to be a function of macrophyte community composition rather than growth per se. We are particularly interested in developing management techniques that consider beneficial as well as negative attributes of submersed aquatic vegetation. Not all submersed macrophytes are adventive in nature. Accordingly, some species are generally preferred over others. In our studies, we have endeavored to examine physiological and morphological characteristics facilitating competitive success among a broad variety of both exotic and native species.

Our studies of macrophyte growth relative to sediment type have indicated a close association between species success and sediment composition (Barko and Smart 1983, 1986, and unpublished). Sediment properties are affected by system age, watershed characteristics, loading rates, and a variety of biological factors. We have investigated effects of macrophyte growth on sediment nutrient availability, and found that nutrient uptake along, aside from changes in nutrient status due to redox change (see Chen, Barko, and Twilley 1987), can have a profound influence on subsequent macrophyte regrowth. In general, macrophytes with well-developed root systems, and in particular those capable of retaining nutrients internally upon shoot senescence, have a competitive advantage over other species as nutrient supplies in sediments become depleted. Macrophyte

succession is linked to changes in macrophyte nutrient retention. 'Weed problems' appear to be in large part a direct result of disturbances, which undermine the successional process of biotic nutrient retention.

We are currently investigating the feasibility of lessening sediment nutrient availability and inorganic carbon availability by both chemical and biological means. Our purpose in these efforts is to set the stage for preferred vegetation that is better adapted to nondisturbed environmental conditions. It is possible that such changes can be perpetuated through replanting programs subsequent to environmental manipulation.

FUTURE RESEARCH

Investigations of the influence of nutritional factors on macrophyte growth will continue, with emphasis on mechanisms regulating inorganic carbon availability in water, and nitrogen availability in water and sediments. In particular, we intend to investigate the role of submersed macrophytes in the nitrogen budget of aquatic systems. These studies will advance our understanding of macrophyte function, and elucidate specific conditions that give rise to proliferation of weedy species in aquatic systems.

Major field studies will continue at Eau Galle Reservoir and the Potomac River. In addition, we are collaborating with the State of Wisconsin, Department of Natural Resources, on studies in Devils Lake, and with the State of South Carolina, Department of Health and Environmental Control, on studies in Lake Marion. Objectives of these field efforts are to determine the influence of macrophytes on the environment, and to validate in nature results of past laboratory studies. Results of this work will ultimately provide guidance for aquatic plant management within an ecological context. The theme of aquatic plant 'control' continues to evolve into one of aquatic plant 'management' as advances are made in understanding the ecology of aquatic macrophyte communities.

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Effects of Growth of Submersed Aquatic Macrophytes on Sediment Chemistry

by
R. L. Chen* and J. W. Barko*

INTRODUCTION

Sediment composition generally plays an important role in the growth and distribution of rooted submersed aquatic macrophytes, which rely primarily on sediment as a source for nitrogen and phosphorus. Availability of these and other nutrients to submersed macrophytes depends on physical and chemical characteristics of the sediment and microbial activity in the rhizosphere. These factors are, in turn, affected by the development of the macrophyte roots in the sediment profile. Macrophyte roots influence sediment redox conditions by transporting oxygen produced in the shoots to the sediment (Jaynes 1985). Changes in sediment redox conditions can affect the availability of nutrients, thus potentially influencing macrophyte growth and successional development in aquatic ecosystems. This study was designed to assess the effects of macrophyte growth on sediment chemistry under controlled environmental conditions in the laboratory.

METHODS AND MATERIALS

Hydrilla verticillata, a submersed macrophytes, and *Sagittaria latifolia* an emergent macrophyte, were grown on fine-grained inorganic sediments from Lake Washington (Washington) and Brown's Lake (Mississippi) in the Waterways Experiment Station greenhouse facility (described in Barko and Smart 1981). General characteristics of these sediments are presented in Table 1.

Table 1
Characteristics of Brown's Lake and Lake Washington
Sediments Used for Laboratory Investigation

Sediment	Texture* percent			pH	Exch. NH_4-N	Available PO_4-P	Organic Matter Percent
	Sand	Silt	Clay				
Brown's L.	5.0	77.0	18.0	7.2	114.2	130.6	5.2
L. Washington	22.0	68.0	10.0	6.9	156.9	150.8	10.4

* Values for sediment texture are single observations. Particle size (texture) of the sediments is determined by the hydrometer method modified by Patrick (1958).

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Growth experiments were conducted in large (1,200-l) fiberglass tanks in a greenhouse facility under partially controlled environmental conditions. Vegetation was maintained under conditions of different light intensity at $25 \pm 1°$ C water temperature in an attempt to provide optimum conditions for growth. Unvegetated and *Sagitarria* treatments were exposed to full sunlight within the greenhouse (approximately 1,500 $\mu E/m^2/sec$ at midday); a 33-percent reduction in sunlight was imposed on tanks containing *Hydrilla* by covering with neutral density shade fabric.

Vegetation was planted in 2-l sediment containers (10 × 10 × 15 cm) at the beginning of the study. Sediment and vegetation samples were collected at initiation of the study and at 2-week intervals thereafter for 8 weeks. Three replicates of each species-sediment combination were removed from culture tanks during each sampling date for physical, chemical, and biomass measurements.

Sediment redox profiles were measured with platinum electrodes coupled with a calomel reference electrode. Platinum electrodes were constructed and calibrated according to the method described by Chen and Keeney (1974). Vertical redox distribution was measured with a special motor-driven assembly which advanced a platinum electrode through the sediment profile (Patrick and DeLaune 1972).

After the sediment redox was determined, aboveground biomass was clipped and removed from the sediment sections. The sediment from these containers was then sectioned horizontally into two equal sections. Three sediment cores (2.54 cm in diameter) were obtained from each section for chemical analyses, and the remaining sediment was used for root mass determination. One sediment core sample from each replicate sediment container was centrifuged at 10,000 rpm for 20 minutes to separate interstitial water. The remaining core samples were chemically extracted with either $1\underline{N}$ KCl (Bremner 1965) or a dilute acid-fluoride solution (Olson and Dean 1965) to determine the concentrations of exchangeable ammonium and available phosphate, respectively. Concentrations of total nitrogen and total phosphorus in macrophytes were determined following digestion of plant tissue samples in a mixture of sulfuric acid and hydrogen peroxide (Allen et al. 1974). Ammonium, nitrate, nitrite, and orthophosphate in the interstitial water and sediment extracts were determined with a Technicon AutoAnalyzer (Ballinger 1979). Concentrations of iron in the interstitial water were determined with an atomic absorption spectrophotometer.

RESULTS

Biomass production

Hydrilla biomass increased from less than 0.01 to approximately 30 g per container on both Lake Washington and Brown's Lake sediments (Figure 1) during a study period of 8 weeks. Almost all the biomass produced was in the aboveground portion since roots comprised less than 2 percent of the total biomass. Changes in biomass of *Sagittaria* were also pronounced, increasing from 10.2 g/container to approximately 60 and 70 g/container in Brown's Lake and Lake Washington sediments, respectively. Root biomass in *Sagittaria* was approximately 30 percent of the total biomass (root plus shoot) at the conclusion of the study.
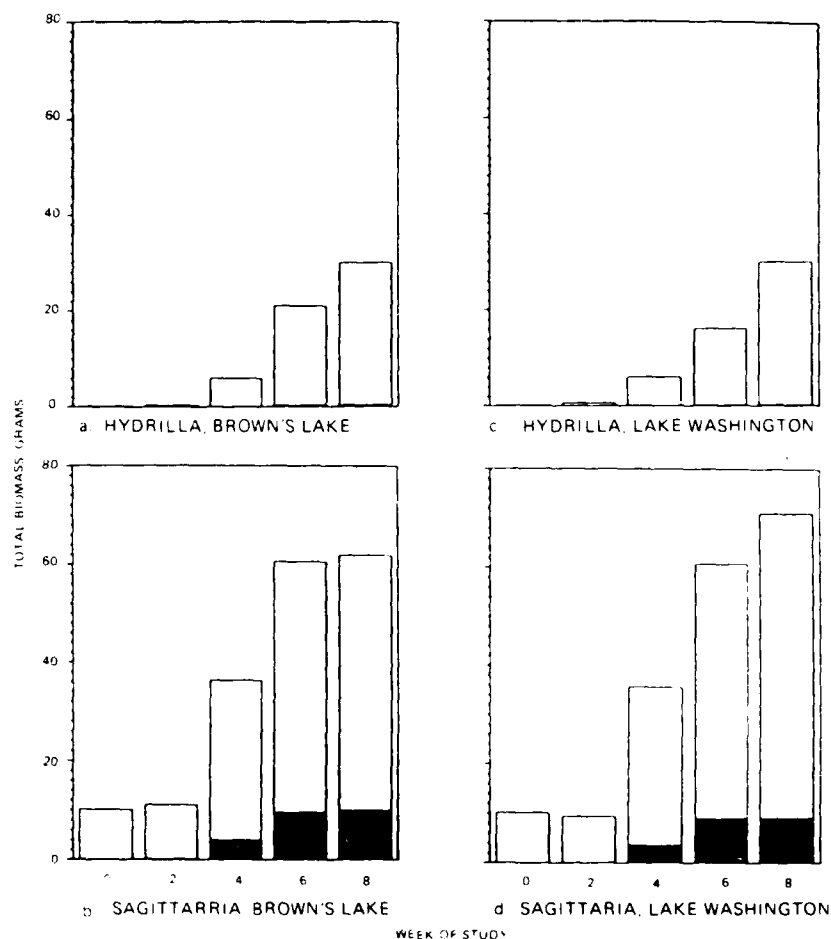
Figure 1. Total aboveground and below-ground portions of biomass produced in the Brown's Lake and Lake Washington sediments during the greenhouse study ($\square$ = aboveground, $\blacksquare$ = below-ground)

Redox potential

The redox potential (Eh) at the sediment-water interface of both sediments ranged from +250 to +300 mV throughout the experiment. On unvegetated sediments, Eh decreased sharply from +250 at the sediment surface to -250 within 1 cm below the surface, and remained low (-250 to -300 mV) with increasing depth. A more pronounced decline in Eh with depth was measured in Lake Washington than in Brown's Lake sediment. Growth of *Hydrilla*, with minimal root mass, did not appreciably affect the vertical distribution of sediment Eh; however, growth of *Sagittaria*, with a relatively massive root system, resulted in substantial oxidation of both sediments within 6 weeks (Figure 2).

Nutrients in interstitital water

Interstitial water ammonium nitrogen (NH_4-N) concentrations in the bottom half of the unvegetated sediment remained constant throughout the experiment, but in the upper layer decreased moderately beyond 4 weeks (Figure 3). Considerable amounts of dissolved ammonium-n were removed from the interstitital water of

Figure 2. Changes in Eh of the Brown's Lake and Lake Washington sediment profile as affected by the growth of *Hydrilla* and *Sagittaria* over an 8-week period. (Determinations made at 6 weeks.)

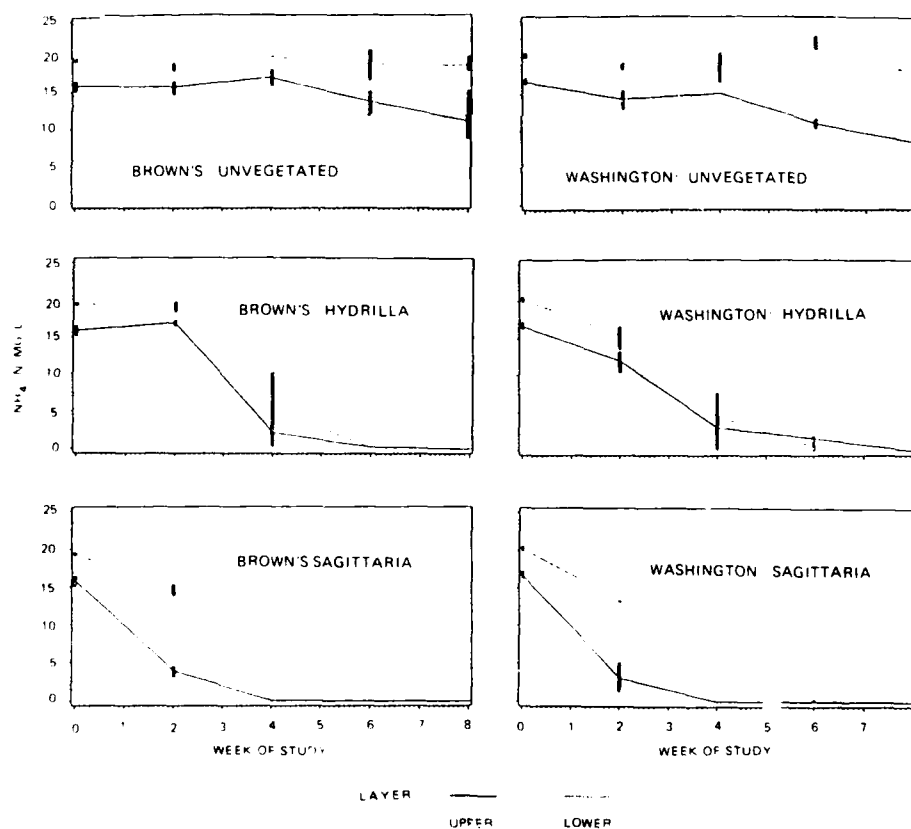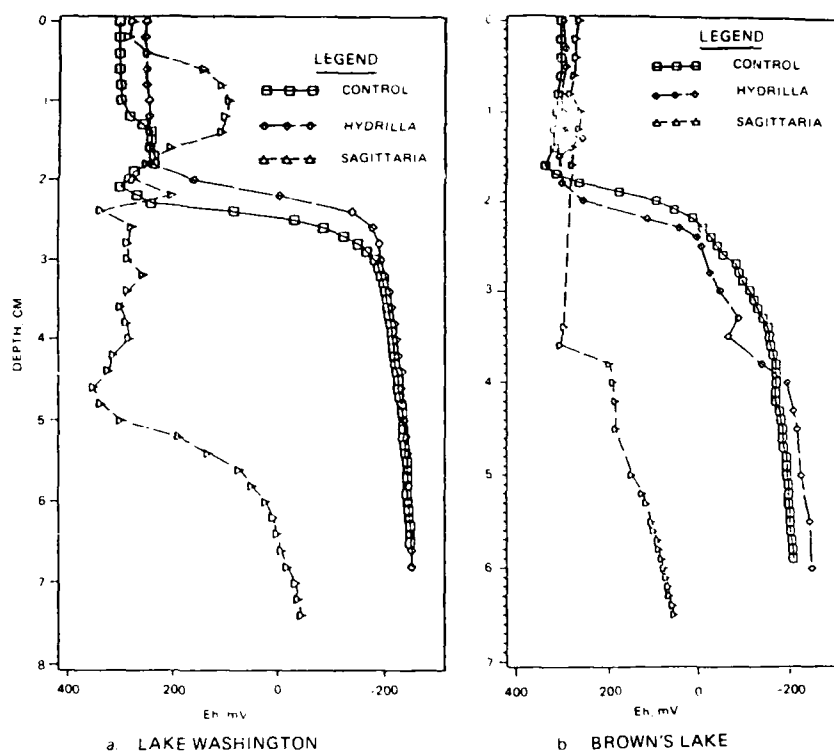a. LAKE WASHINGTON

b. BROWN'S LAKE

Figure 3. Changes in interstitial water ammonium-N concentration (milligrams/litre) in the Brown's Lake and Lake Washington sediments during the greenhouse study. (Values are mean ± 1 standard error; N = 3.)

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both sediments by both macrophyte species beyond about 2 weeks of growth. By the end of the study, concentrations of interstitial ammonium-n in all vegetated sediments were completely exhausted. Concentrations of the oxidized forms of inorganic nitrogen (nitrate plus nitrite-N) increased over the last 2 weeks of study from undetectable levels initially to 0.05 mg/l in vegetated Brown's Lake sediment and 0.15 and 0.25 mg/l in Lake Washington sediment, vegetated with *Sagittaria* and *Hydrilla*, respectively (Figure 4).

Orthophosphate (PO_4-P) concentrations in the interstitial water of Brown's Lake sediment fluctuated during the experiment and were not consistently affected by the growth of either *Sagittaria* or *Hydrilla*. In contrast, there was a consistent decline in orthophosphate concentrations in Lake Washington sediment. This decline was most pronounced in the vegetated sediments where orthophosphate was reduced at the end of 8 weeks from 0.45 to <0.01 mg/l by the growth of *Sagittaria* and from 0.45 to 0.03 mg/l by *Hydrilla* (Figure 5).
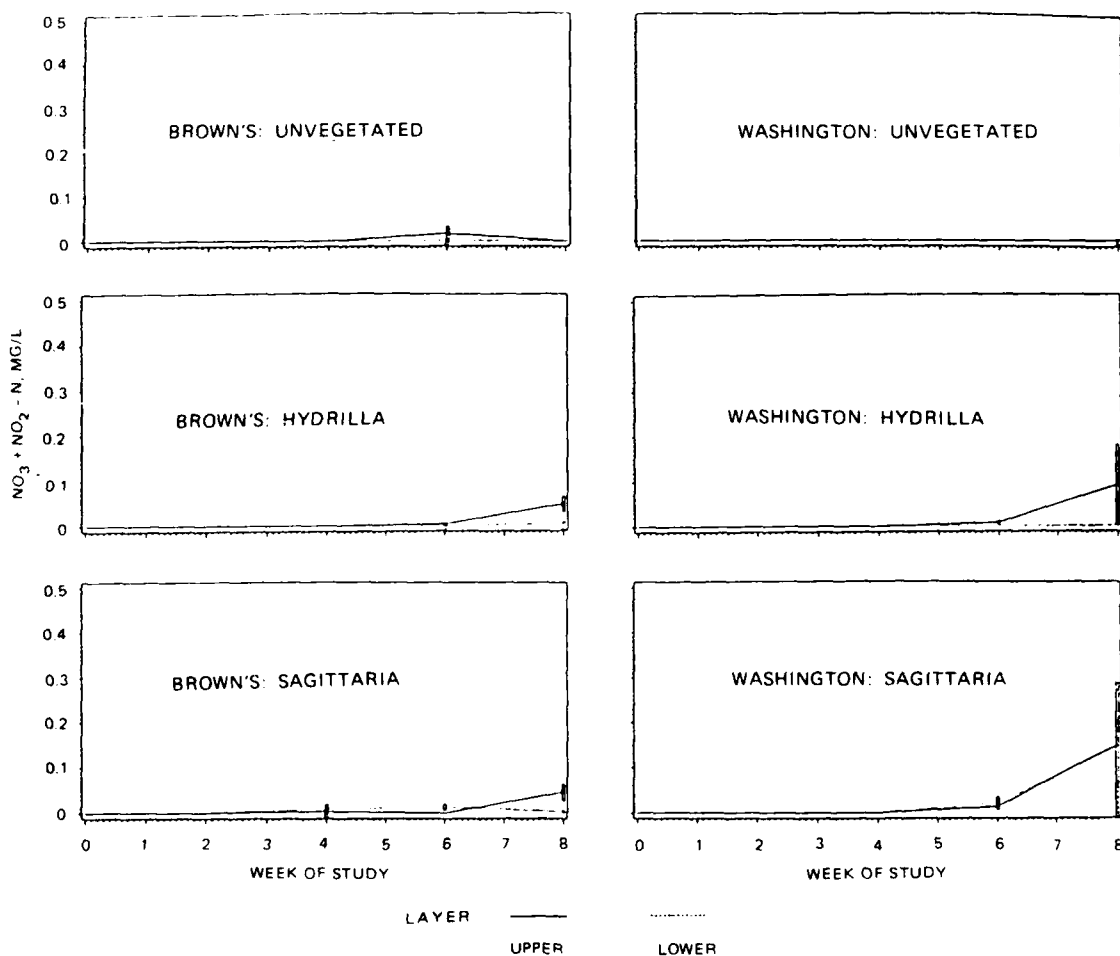

Figure 4. Changes in interstitial water nitrate plus nitrite-N concentration (milligrams per litre) in the Brown's Lake and Lake Washington sediments during the greenhouse study. (Values are mean ± 1 standard error; N = 3.)
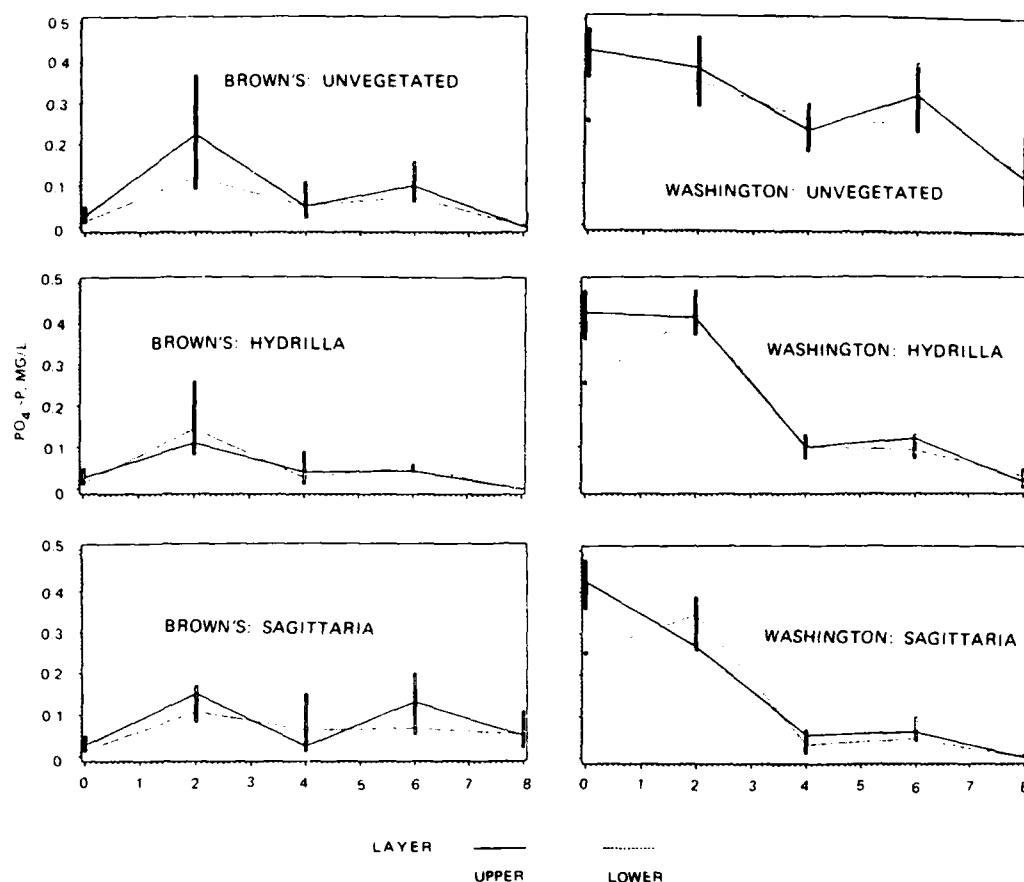
Figure 5. Changes in interstitial water orthophosphate concentration (milligrams per litre) in the Brown's Lake and Lake Washington sediments during the greenhouse study. (Values are mean ± 1 standard error; N = 3.)

Dissolved iron in the interstitial water of unvegetated sediments and in sediments vegetated with *Hydrilla* remained essentially unchanged during this study (Figure 6). However, in containers vegetated with *Sagittaria*, dissolved iron declined in Brown's sediment and increased in Lake Washington sediment during the study period. Differences in iron concentrations between upper and lower sediment strata were minor except for *Hydrilla* on Brown's Lake sediment and *Sagittaria* on Lake Washington sediment, where iron concentration was consistently greater in the lower stratum.

Exchangeable sediment nutrients

Changes in concentrations of exchangeable ammonium-n and available orthophosphate in unvegetated sediments were minor throughout the experiment (Figures 7 and 8); however, exchangeable ammonium-n did decline somewhat in the upper layer of Brown's Lake sediment toward the end of the study. In contrast, relatively substantial declines in the concentrations of exchangeable ammonium-n and available orthophosphate occurred in both vegetated sediments. The decline in available orthophosphate was far less precipitous, and occurred somewhat later in the study than did the decline in exchangeable ammonium-n in both vegetated sediments.
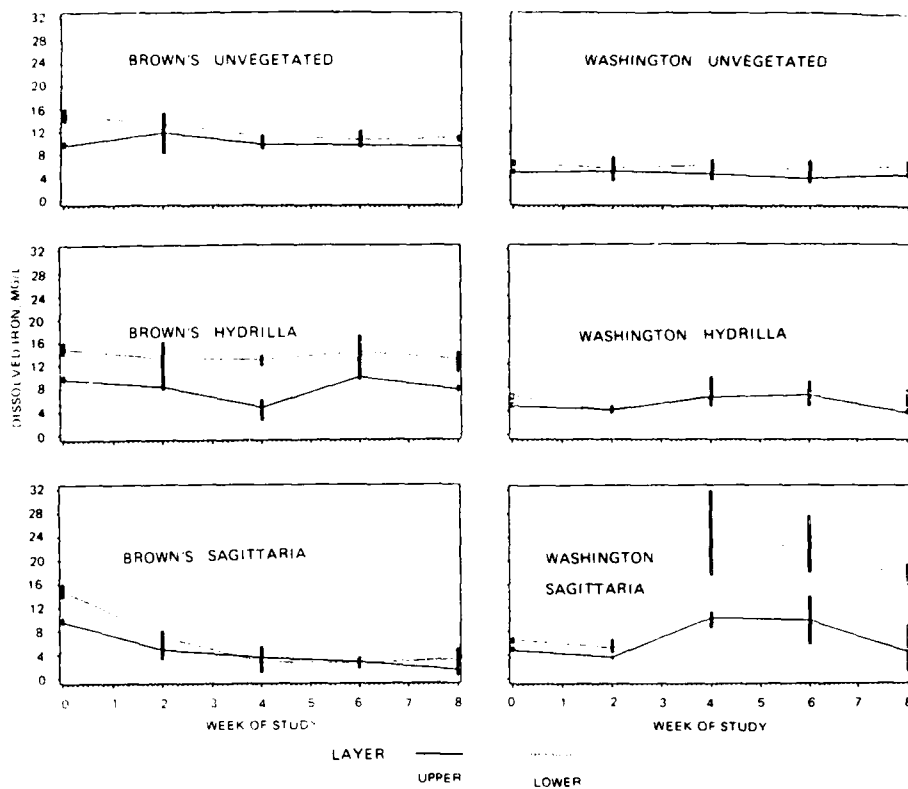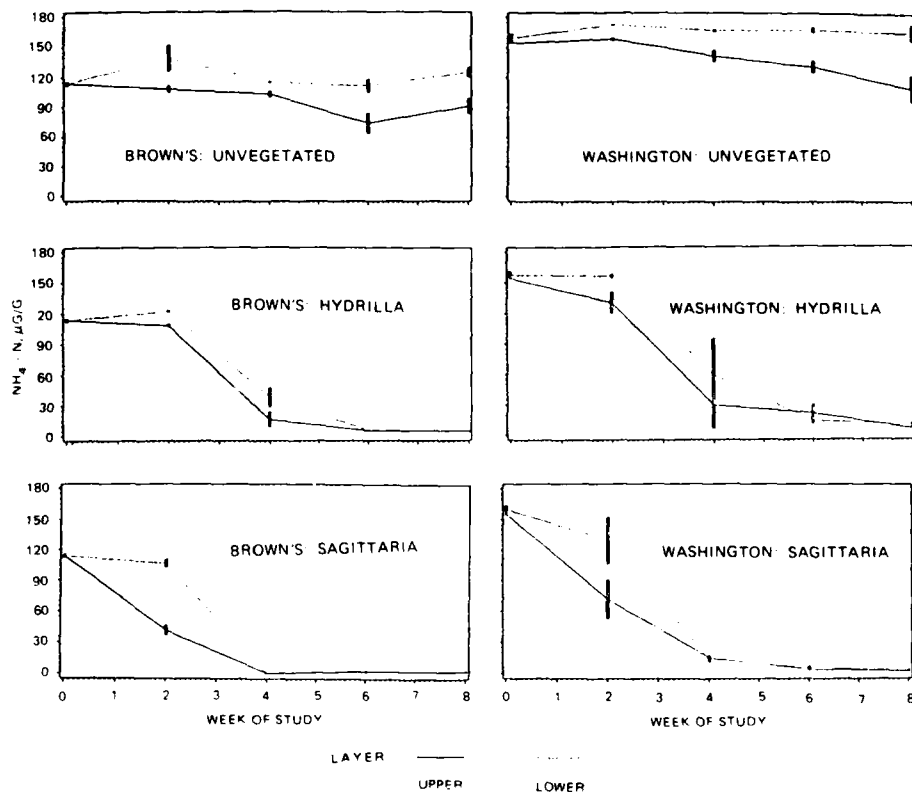
Figure 6. Changes in interstitital water-soluble iron concentration (milligrams per litre) in the Brown's Lake and Lake Washington sediments during the greenhouse study. (Values are mean ± 1 standard error; N = 3.)

Figure 7. Changes in exchangeable ammonium-N concentration milligrams per gram) in Brown's Lake and Lake Washington sediments during the greenhouse study. (Values are mean ± 1 standard error; N = 3.)
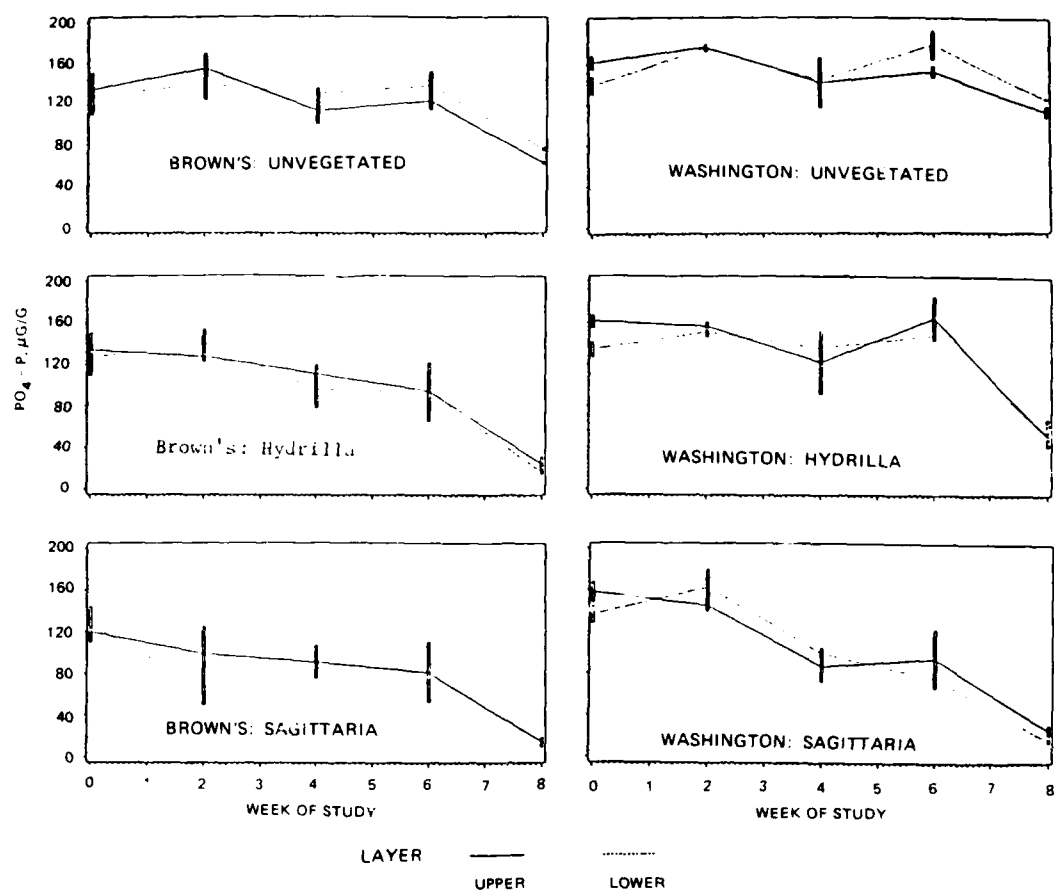
Figure 8. Changes in available orthophosphate concentration (milligrams per gram) in Brown's Lake and Lake Washington sediments during the greenhouse study. (Values are mean ± 1 standard error; N = 3.)

DISCUSSION

Biomass production in both species, expressed on the basis of sediment surface area (approximately 2.1 kg/m² for *Hydrilla* and 4.5 kg/m² for *Sagittaria*), was much greater than that which normally occurs in the natural environment. Thus, the effects of submersed aquatic vegetation on sediment chemistry reported here need to be viewed as representative of maximum potential.

An increase in redox potential of vegetated sediments, particularly with *Sagittaria*, with absence of any redox increase in unvegetated sediments indicates that oxygen was transported from shoots to roots and diffused into the sediment. A significant increase in the thickness of the oxidized layer of the sediment in the case of *Sagittaria* as opposed to *Hydrilla* suggests that root mass may play an important role in affecting change in sediment redox potential.

It appears that much of the decrease in sediment nitrogen has resulted from plant uptake. Upward diffusion of soluble ammonium and/or concurrent nitrification-denitrification reactions may also have accounted for some losses of sediment nitrogen. Changes in redox potential from reduced to oxidized conditions

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in sediment vegetated with *Sagittaria* may have established conditions favorable for nitrification. This is suggested by increased concentrations of nitrate- and nitrite-N noted in the surficial layers of vegetated sediments.

Changes in sediment redox potential and phosphorus concentration gradients due to plant growth and uptake can greatly affect the equilibrium between the interstitital water and the sediment particles, and thus potentially alter the release rate of phosphorus from the sediment into the overlying water. Desorption of phosphorus from the Brown's Lake sediment into the interstitial water was probably regulated by phosphorus (P) uptake rates of the aquatic macrophytes, since only minimal dissolved phosphate was detected in the interstitital water of this sediment. On Lake Washington sediment, plants had access initially to relatively greater concentrations of P in the interstitital water. Nevertheless, most of the P taken up by plants from either sediment in this study appears to have been derived by desorption from particulate pools as suggested by Barko and Smart (1980).

Solubility of many metals in sediment is controlled by sediment redox, sediment pH (Gotoh and Patrick 1974), and metal complexation (Lindsay 1979). Sediment oxygen demand and the magnitude of oxygen release by plant roots may influence nutrient availability in the sediment. Local precipitation of ferric oxides caused by elevated Eh around *Spartina* roots has been reported by Mendelssohn and Postek (1982). In contrast with this finding, however, we noted an increase in dissolved iron concentration associated with redox increase in Lake Washington sediment vegetated with *Sagittaria*. Increases in dissolved iron during the growth of *Halodule* in iron-rich anaerobic marine sediment was reported by Pulich (1982). The relatively high dissolved iron concentrations in the lower layer of Brown's Lake sediment planted with *Hydrilla* and the large accumulation of dissolved iron in Lake Washington sediment planted with *Sagittaria* were likely caused by formation of metal-organic complexes resulting in dissolution of redox-sensitive metals such as iron in vegetated sediments. Alternatively, changes in pH resulting in increased solubility of iron in the rhizosphere may have occurred (Berthelin and Boymond 1978). The mechanism responsible for the increase in dissolved iron in the vegetated sediment deserves further study.

ONGOING STUDIES

Field studies are being planned for conduct in Eau Galle Reservoir, Wisconsin, and in the Potomac River, Washington, DC, to provide field verification of the effects of aquatic macrophytes on sediment chemistry. Future laboratory research activities will be directed toward examining the effects of macrophyte growth on the magnitude and rates of nitrogen transformations in water and sediments.

ACKNOWLEDGMENTS

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Effects of Submersed Aquatic Plants
on Their Environment

by
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INTRODUCTION

Aquatic macrophytes affect their environment in many ways. In fact, components of their metabolism are likely to have important and far-reaching effects on the distribution of aquatic animals, including fishes. Phytoplankton in the water of the plant beds and algae attached to macrophyte shoots contribute to littoral community metabolism and enhance the overall productivity of aquatic ecosystems. The value of aquatic systems to many users depend at least in part on the productivity, type, and distribution of vegetation. Due to the complex interacting processes of element uptake, precipitation, and release, water chemistry within macrophyte beds is often substantially different from that in outlying open water. Aquatic macrophytes play a significant, but as yet poorly understood, role in the cycling of elements and in sedimentation in aquatic systems.

Present understanding of relationships between aquatic macrophytes and the quality of the environment is quite poor owing to past bias toward predominantly open-water processes and to real difficulties in sampling within the littoral environment. During the past year, we have expanded our efforts to better understand the influence of environment on macrophyte growth to include an assessment of how macrophytes themselves influence the environment.

As part of an extensive project dealing with the influence of aquatic macrophytes on their habitat, a study was conducted during the summer of 1986 in Eau Galle Reservoir to assess the impact of littoral macrophyte vegetation on physical and chemical characteristics of the surrounding water over daily cycles. This study was implemented concurrently with investigations on the biota (epiphytes, phytoplankton, zooplankton, and benthic invertebrates) associated with the macrophytes and thus potentially affected by macrophyte-influenced conditions of the microenvironment.

METHODS AND MATERIALS

Eau Galle Reservoir is a small flood-control impoundment in west-central Wisconsin (Pierce and St. Croix Counties). It has a surface area of 60 ha, maximum depth of 9 m, and mean depth of 3.2 m. Its four tributaries, the largest of which

is the Eau Galle River, provide enough water for the reservoir to have a turnover time of only 0.07 year. The drainage basin of 166 km_2 is predominantly wooded and farmland. Detailed descriptions of the reservoir are available in Kennedy (1985) and Kennedy and Gunkel (in preparation).

While the littoral regions of the reservoir are not particularly extensive for its size, Eau Galle Reservoir has abundant submersed aquatic vegetation in its shallow waters (Filbin and Barko 1985; Godshalk and Barko, unpublished data). *Potamogeton nodosus* and *Ceratophyllum demersum* are by far the dominant species, with densities as high as 500 g dry weight/m_2 during the study period; *Potamogeton foliosus*, *Potamogeton pectinatus*, *Heteranthera dubia*, *Najas flexilis*, and *Elodea canadensis* are also present. The littoral vegetation is quite typical of that of most north-temperature, hardware, eutrophic lakes.

Diel sampling was conducted at three littoral sites in Eau Galle Reservoir (Figure 1); a fourth site was located in deep water near the center of the lake to allow for a comparison of littoral versus pelagic characteristics and processes. Each of the littoral sites had a depth of ca. 1.6 m and was marked by a metal
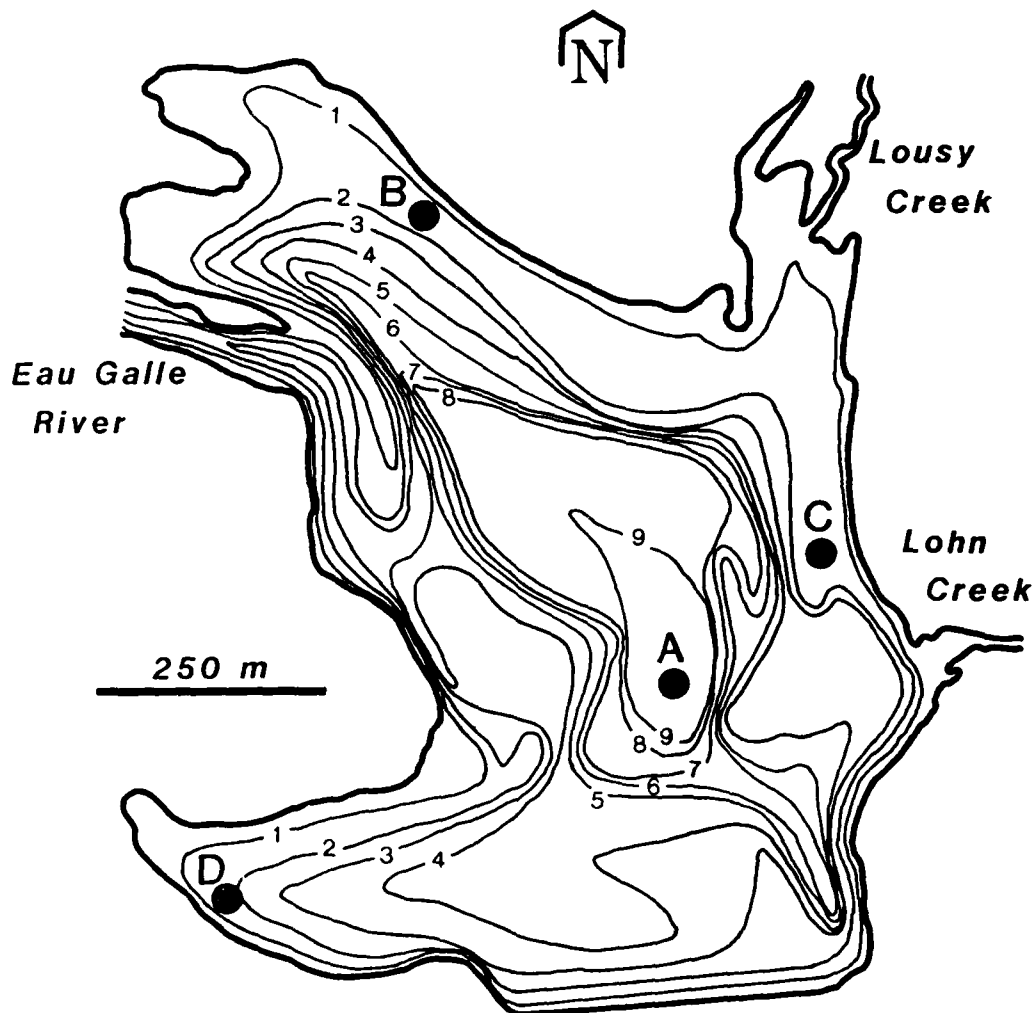

Figure 1. Morphometric map of Eau Galle Reservoir with depth contours (1-m intervals) and diel sampling sites (A, B, C, D) indicated

fencepost driven into the sediment. Site B (in the mouth of the northwest bay) was the least densely colonized site with respect to macrophyte biomass throughout the growing season. It was also the site closest to the edge of the littoral zone and, therefore, subject to greater mixing with water from the pelagic zone of the reservoir. Sites C (just north of the mouth of Lohn Creek) and D (well into the protected southwest bay) had much denser macrophyte stands and were physically removed from open-water influences. In situ measurements were made biweekly; water samples for chemical analysis in the laboratory were collected monthly. Sampling was intentionally performed during clear, calm weather to maximize the potential diel differences in parameters measured.

On the day before each sampling run, a plastic pipe (CPVC 1.5-in. ID) was slipped over the fencepost with minimal disturbance to the adjacent vegetation. The pipe was equipped at intervals of 30 cm with horizontal ports (opening, 3.5-mm in diameter) attached to Tygon R3603 tubing through the pipe to the surface. The six tubes from the ports were fastened together and extended 6 m downwind of the sampling site. The ends of the tubes were kept at the surface with a small buoy. The midlake site was sampled with a similar apparatus suspended from a boat at each time of sampling.

Samples were taken at each site three times during a 24-hr period: just before dawn ("morning"), "midday," and just before dusk ("evening"). The workboat slowly approached each sampling station from the downwind side and was moored just close enough so that the tubing from the sampling ports could be attached to peristaltic pumps on the boat. Water was pumped through each tube for 1 min, which was determined previously to provide complete flushing of the sampling tube. Then, enough water was pumped to fill a 0.5-l brown plastic bottle that was kept cold until returned to the laboratory (2-hr maximum). After pumping, the boat was pulled to the sampling site, and the sonde unit of a Hydrolab Surveyor II was lowered in 30-cm intervals to measure in situ temperature, pH, dissolved oxygen, and conductivity. The Hydrolab unit was calibrated at least daily to standard buffers and solutions of known conductivity and by Winkler titration for dissolved oxygen, according to the manufacturer's instructions.

The bottled sample was vacuum-filtered (<200 mm Hg) in the laboratory through 0.45-μM (micron) pore size cellulose nitrate membrane filters (Millipore HA or Micro Filtration Systems A045A). Filtered water was assayed for ammonium using Nessler reagent American Public Health Association (APHA) 1975, Method 418 B) and for soluble reactive phosphorus (APHA 1975, Method 425 F). An aliquot was acidified (pH < 2) with nitric acid and later analyzed for Na^+, K^+, Ca^{++}, and Mg^{++} by flame photometry.

At the biweekly midday sampling, additional water samples integrated over the top 1.25 m of the water column with a pipe sampler (Barko et al. 1984) were split into two portions. One portion of the integrated sample was vacuum-filtered, as described above, through glass fiber filters (Micro Filtration Systems GF75 or Gelman Type A/E), which were then frozen until analyzed for chlorophyll a (Wetzel and Likens 1979). The second portion of the midday integrated sample was preserved with Lugol's solution (APHA 1975) for later enumeration of algae.

RESULTS

Temperature

On all sampling days, water was nearly isothermal from the surface to the 1.5-m depth at the open-water site and exhibited slight diel changes in temperature (Figure 2a). Early in the growing season (i.e., May), when there was little macrophyte biomass, littoral zone sites showed similarly uniform heating of water at all depths each day. However, the littoral sites often showed diel temperature fluctuations greater than 5° C in contrast to the 2° to 3° C of daily warming and cooling observed in open water. At midday, after several hours of direct solar radiation with little mixing, there was a temperature gradient of up to 5° C from the surface to the bottom (Figure 2b) at the littoral sites.

By midsummer (July), when littoral vegetation was approaching peak seasonal standing crop, depthwise temperature gradients were much steeper, as great as 9° C change from the surface to the 1.5-m depth (Figure 2c). These gradients were more persistent, and the temperatures at any given depth were more constant
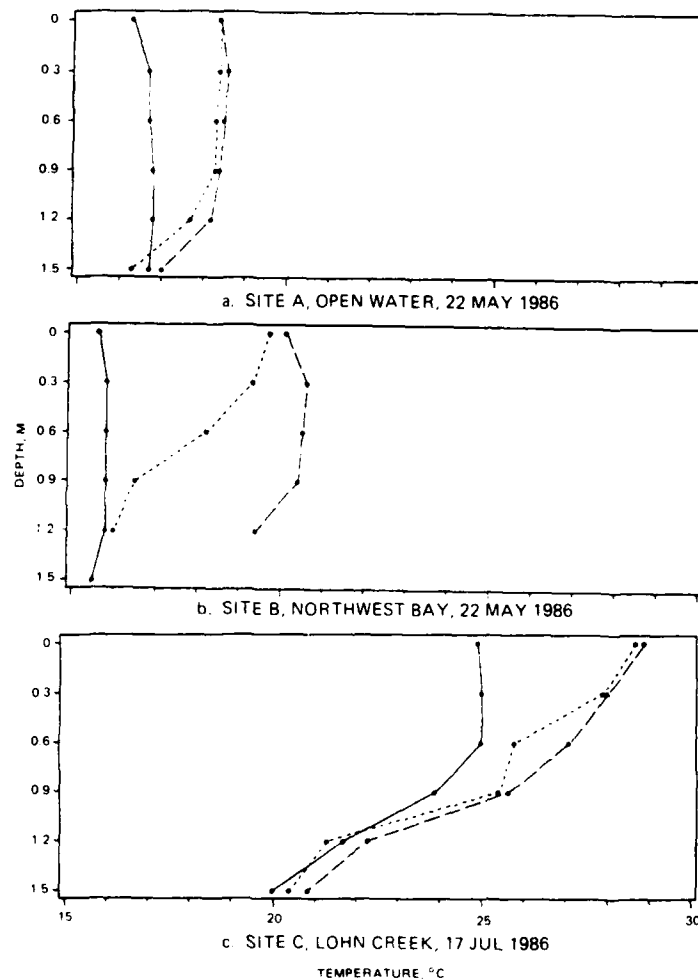

Figure 2. Diel temperature data (solid line, morning; dotted line, midday; broken line, evening)

over a 24-hr period than earlier in the season. While the water above 0.9-m depth did undergo a daily heating/cooling cycle of 2° to 4° C, water near the bottom was essentially protected from changes in temperature due to shading by the plants and their prevention of heat transport by water movement.

Dissolved oxygen

In the spring, at the littoral sites there was little variation in oxygen concentrations either with time of day or with depth: the water at all depths uniformly increased in oxygen content during the day (Figure 3a), becoming slightly supersaturated. This response was not different from that observed in the open-water site throughout the study period, and hence, is attributed to metabolic activity of phytoplankton on a diel cycle.

During the summer, highest oxygen values usually were found at the surface, with secondary peaks occurring closer to the bottom (Figure 3b). Sometimes, however, highest oxygen concentrations and the greatest daytime increases in dissolved oxygen occurred at middepths. The presence of middepth oxygen maxima seemed to vary in relation to density of macrophytes. The middepth oxygen peaks appeared as macrophyte abundance increased, but they disappeared late in the season near the time of mass macrophyte senescence. Subsurface peaks in oxygen concentration occurred at the sites most densely colonized with macrophytes (Sites C and D) as early as June. In July, high concentrations of oxygen below the surface were measured at Site D again, and for the first time, at the less densely colonized Site B. The macrophytes had their densest canopies at Site C in July, and at all littoral sites thereafter. Oxygen concentrations then were highest at the surface and declined steadily with depth. During late summer, dense mats of filamentous algae developed on the surface of the macrophyte beds, coincident with macrophyte senescence.

Throughout the summer, morning samples showed an increasingly high gradient of oxygen concentration and degree of saturation with depth indicative of an acute demand for oxygen at the bottom. The nighttime respiratory demand for oxygen at all depths, but especially near the bottom, was pronounced by midsummer (Figure 3c).

pH

The littoral sites early in the season and the open-water site throughout the study incurred only slight increases in pH during daylight (Figure 4a). These increases in pH occurred quite uniformly at all sampled depths, and usually reached their greatest extent, at least in the upper waters, by midday. As the macrophyte standing crop increased, diel changes in pH increased significantly, especially at depths close to the surface (Figure 4b). By late summer, highest pH values were measured at depths near 0.5 m (Figure 4c).

Conductivity and cation concentrations

Like other parameters, conductivity behaved in the littoral sites in the spring as it did all summer in the open water: there was little change with time of day or with depth (except for the expected slight increases very near the bottom).

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a. 22 MAY 1986

Figure 3. Profiles of oxygen saturation in the southwest bay (Site D) at different times of the day (solid line, morning; dotted line, midday; broken line, evening)

b. 19 JUNE 1986

c. 17 JULY 1986

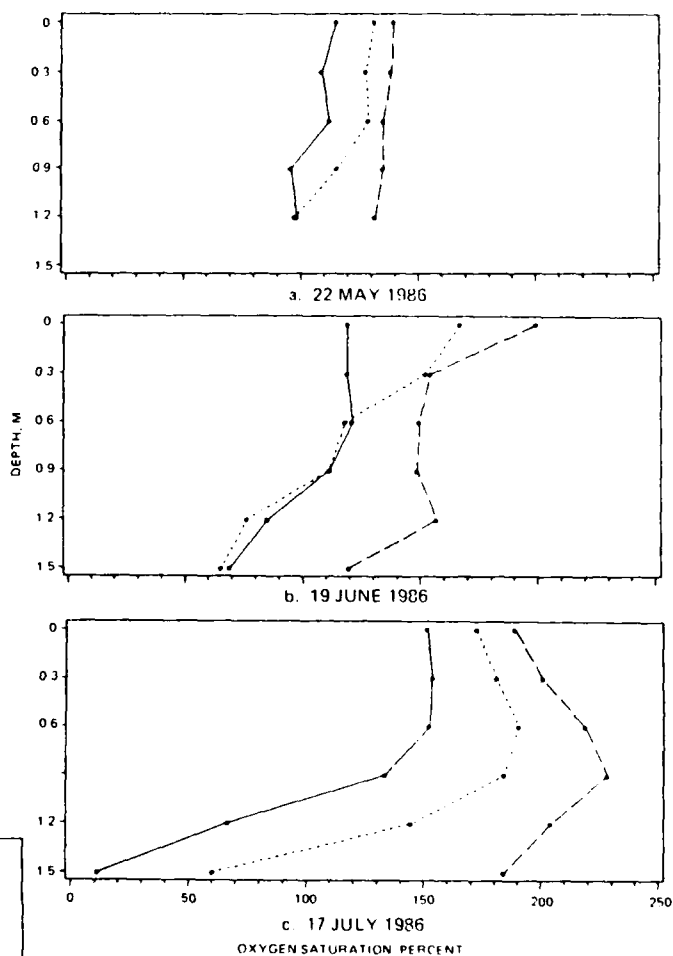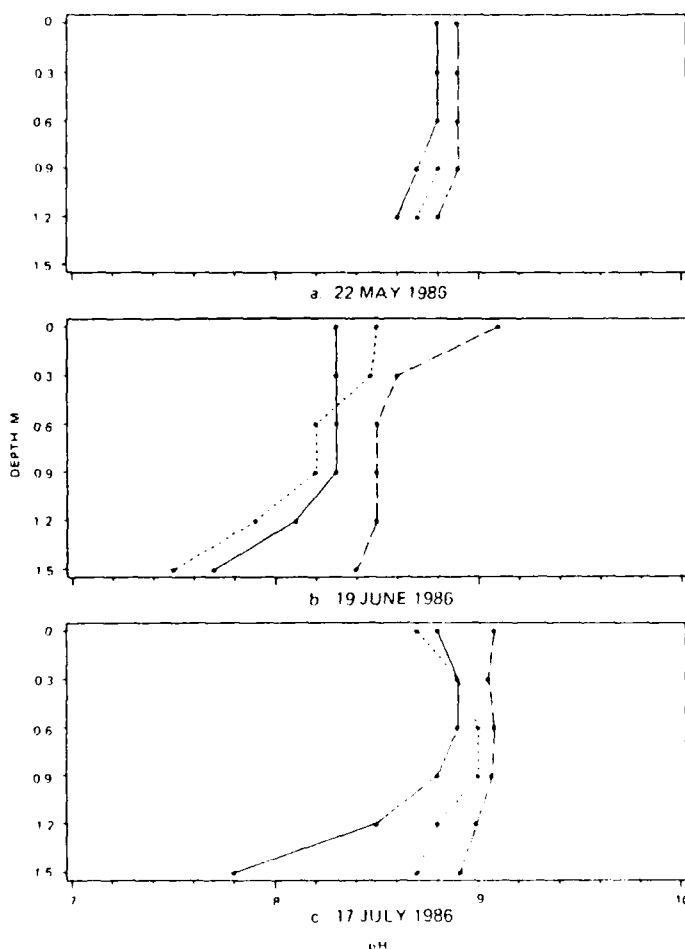OXYGEN SATURATION PERCENT

a. 22 MAY 1986

b. 19 JUNE 1986

Figure 4. In situ measurements of pH in the southwest bay (Site D) on different dates over a diel cycle (solid line, morning; dotted line, midday; broken line, evening)

c. 17 JULY 1986

pH

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Later in the season, surface waters at all sites, including the open water, showed minor declines in conductivity from morning to evening (usually <10 μS/cm from the normal value of ca. 230 μS/cm). Concentration of Na+, K+, and Mg++ exhibited no discernible trends over time or season, but generally increased at the lowest sampling depth. Ca++ content quite consistently decreased, but only to a minor extent, from sampling to evening in surface waters.

Nitrogen and phosphorus

As a rule, ammonia-N and soluble reactive P were barely detectable, although concentrations of both increased in the water just above the sediments. Such increases were particularly great (up to 11 mg NH^3-N/l and nearly 0.2 mg P/ l when it was visibly apparent that the sampling pumps had taken some particulate materials off the sediment surface with water through the lowest part. Trends over time of day and season were not observed.

Suspended chlorophyll

Early in the study period (through 19 June) there were no trends among sites in amounts of chlorophyll suspended in the top 1.25 m of water at each sampling site, all measured concentrations being about 5 μg/l. Late in the growing season (17 July and later) when phytoplankton blooms were occurring in the open water, pelagial concentrations of chlorophyll suspended in the surface waters were up to fivefold greater than at littoral sites (Table 1). Site B in the northwest bay had considerably greater chlorophyll concentrations in the water among the macrophytes than did the other two littoral sites; this was because Site B was less densely colonized with vascular plants, closer to open water, and therefore more capable of supporting a local phytoplankton population and more susceptible to influx of phytoplankton from pelagial waters.

Table 1

Measurement of Chlorophyll a (μg/l)
Suspended in the Water Column at
Each of the Diel Sampling Sites at
Midday

	Site			
Date	A	B	C	D
22 May	3.5	3.6	4.5	2.6
5 June	5.6	6.2	4.2	6.7
19 June	2.3	2.7	1.5	3.1
3 July	27.9	31.3	2.1	11.0
17 July	52.0	38.5	9.2	20.7
1 August	78.1	52.6	39.1	38.0

DISCUSSION

The most apparent effect of macrophyte vegetation on environmental characteristics of the littoral zone is resistance to mixing (i.e., water circulation) afforded by the sheer mass of the plant material. Impedance of water movements

by macrophytes has been measured previously (Madsen and Warncke 1983). Effects of aquatic plants on temperatures in the littoral zone are passive; they do not depend on the metabolic activities of the plants themselves.

Open water in Eau Galle Reservoir, with no obstructions to mixing, heated and cooled quite uniformly with depth. The proximity to shore, especially in more protected bays, decreased the exposure of littoral waters to wind in the afternoons and evenings, and as a result, the shallower waters gained more heat each day in the spring even without the effect of macrophytes. During the spring, both the open-water and littoral sites exhibited nightly cooling to nearly the same extent as daytime warming. Other investigations (e.g., Kersting 1983) have similarly found that thermal stratification established in shallow waters without macrophytes breaks down with evening cooling and wind activity. Thus, at this time of the year, the significant gradients of temperature in Eau Galle Reservoir were in time (i.e., hours), not in depth.

As the growing season progressed, diel fluctuations in temperature diminished at the littoral sites while steep gradients of temperature with depth became established. Macrophytes prevented mixing, and the gradients were protected. Thus, organisms found in the bottom of the densely vegetated littoral zone did not experience rapidly or greatly fluctuating temperatures; however, if those organisms moved vertically, they would have encountered extreme changes in temperature, up to nearly 10° C per 1.5 m.

The concentrations of dissolved oxygen in the waters of dense plant stands were very much influenced daily and seasonally by the plants' photosynthetic production (daytime) and respiratory depletion of oxygen (nighttime and daytime). Early in the season, the middepth oxygen peaks in the littoral zone of Eau Galle Reservoir probably represented the sites of greatest photosynthetic activity of macrophytes, i.e., at the top of the developing shoots. By midsummer (July), the macrophytes had reached the surface, yet there remained greater evening oxygen concentrations near the middle of the water column than at either the surface or the bottom.

There are at least two potential causes of middepth oxygen maxima, and they were probably acting in combination. First, since the macrophytes, largely *Ceratophyllum*, were already undergoing some senescence below the canopy, as evidenced by leaf loss and color change, the high oxygen concentrations well below the water's surface may have been partly created by photosynthetic activity of increasing populations of epiphytes. These algae could have been stimulated by nutrients lost from the senescing portions of vascular plants or by nutrient diffusion upward from the sediments. If the epiphytes subject to this fertilization were still high enough in the water column to receive adequate light, photosynthetic oxygen production at middepth may have been stimulated. Second, gas exchange was likely occurring across the interfaces between air, water, and sediment. Oxygen gradients established by the plants were made potentially even steeper by loss during the day of oxygen to the atmosphere. During the night, the atmosphere probably served as a source rather than as a sink, with oxygen reentering the surface water. Since bottom sediments have large biological (and chemical) demands that consume molecular oxygen, they likely served as a sink for oxygen during both day and night.

Rapidly declining oxygen concentrations with depth and diel changes in oxygen concentration have been observed in a variety of productive littoral and pelagial aquatic systems (e.g., Reddy 1981, Melack and Fisher 1983). Very steep gradients of oxygen have serious implications for other organisms associated with the macrophytes in the littoral zone. Benthic organisms in Eau Galle Reservoir were exposed daily in summer to changes in oxygen saturation from about 10 percent to nearly 200 percent. Organisms that cannot tolerate several hours of low oxygen concentrations may be required to migrate vertically despite exposure to radically different temperatures; they would have to ascend only 0.5 m to enter water that was fully saturated with oxygen.

Changes in hydrogen ion concentrations during this study also demonstrate the importance of photosynthesis in modifying the chemical environment of the littoral zone. The midday peak of pH commonly observed in surface waters of Eau Galle Reservoir probably indicated a slowdown of net photosynthesis in early afternoon. Increases in pH associated with dense macrophytes in lentic waters have been observed previously (Reddy 1981, Halstead and Tash 1982).

The greatest diel changes in pH in July were usually at the bottom and at the top of the water column. Decrease in pH is readily explained by the acidification associated with respiratory processes of both autotrophs and heterotrophs. This decline in pH at the water surface due to respiration was overcompensated in daytime by even larger increases in pH due to phytosynthesis. Again, gas exchange was probably a contributing factor to the diel variability of pH. Carbon dioxide, removed photosynthetically concomitant with a pH increase at the surface in daytime, might be partly replenished by input from the atmosphere in addition to respiration at night, causing a decline in pH.

SUMMARY AND CONCLUSIONS

Macrophytes in Eau Galle Reservoir play both passive and active roles in influencing the physical and chemical attributes of their habitat. The macrophytic biomass presents a substantial impediment to water movement. Therefore, mixing of surface-heated water to lower depths in the littoral zone is reduced as the number of macrophytes increases during the growing season. Photosynthesis significantly alters the profiles of oxygen and pH within the thermally stratified littoral zone as compared to surficial open waters.

Aquatic macrophytes alter the gradients of environmental parameters in the littoral zone in two ways. First, depthwise gradients are steepened. The normal trends of declining light, temperature, dissolved oxygen, and pH that prevail in pelagial waters exist in littoral waters as well. But, because of the shallow depth of the water column over which changes in the parameters' values occur, the gradients are much steeper. Organisms that live at the union of favorable values along opposing gradients, e.g., epiphytic algae with respect to light and nutrients, may be restricted to a particular narrow depth stratum. Secondly, gradients in time are also made much steeper. Thus, migrational behavior may be a requisite for survival of animals associated with macrophytes.

The steepening of environmental gradients over depth and time adds significantly to the complexity of the littoral zone. As long as conditions are conducive to life, e.g., respiratory processes do not consume all dissolved oxygen, a large variety of organisms can be expected to live associated with macrophytes, exploiting the littoral zone which has much greater diversity and areal productivity than the pelagial zone in the same lake or reservoir.

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The Habitat Value of Submersed Aquatic Vegetation

by
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INTRODUCTION

Aquatic macrophytes comprise an integral part of freshwater systems and influence biological, physical, and chemical conditions. They are a dynamic component of the environment, with biomass and areal cover changing seasonally and in reponse to climatological events. The physical presence of stems, leaves, and roots affects currents, water depths, and deposition and erosion of sediments. Aquatic macrophytes also create structural complexity within habitats by providing refuge and substratum for a wide variety of organisms, including snails, aquatic insects, and protozoans (Pennak 1953).

The increased density and diversity of invertebrates associated with aquatic plants have beneficial effects on fish. Killgore (1979) reported that largemouth bass and other game species were usually concentrated in *Hydrilla* beds located in shallow water. Holland and Lester (1984) found that average catches of northern pike from areas with submersed vegetation were more than 10 times greater than those from sites with no vegetation. Laughlin and Werner (1980) reported that numbers of small-sized longear sunfish and bluegill were positively correlated with height of vegetation and that few adults of either species used areas devoid of aquatic plants. The presence of aquatic vegetation can directly influence fish reproduction. Fish that broadcast their eggs over aquatic vegetation or tree roots include northern pike, carp, goldfish, and golden shiner. While next builders (sunfishes, largemouth bass, crappie, rock bass, warmouth, bowfin, and most bullheads) sometime lay eggs on mud, sand, or silt, they usually choose sites with vegetation (Lagler, Bardaih, and Miller 1962).

While the physical presence of vegetation can enhance aquatic habitats, an overabundance of plants can have negative effects. If structure is too dense, predators cannot easily find and capture their prey. When fish are unable to capture prey readily, their growth rates and physical conditions are adversely affected. Colle and Shireman (1980) reported that the condition of harvestable-sized largemouth bass was affected when *Hydrilla* density exceeded 30-percent coverage. Wiley et al. (1984) reported that optimal macrophyte standing crop was no more than 52 grams dry weight/sq m in central Illinois ponds dominated by pondweed (*Potamogeton crispus*) and bushy pondweed (*Najas flexis*).

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Aquatic plant control is often necessary in lakes, ponds, and rivers when recreation, navigation, or water supply purposes are adversely affected by an overabundance of vegetation. However, cost-effective and environmentally sound plant management programs must take into consideration the value of vegetation in maintaining the physical, biological, and chemical integrity of aquatic systems. Reservoir managers, planners, and engineers can manage aquatic vegetation to achieve project purposes without negatively affecting ecological, recreational, and aesthetic values.

The purpose of this paper is to describe the objectives of a new study, part of the Aquatic Plant Control Research Program, concerning the habitat value of aquatic plants. In addition, preliminary results of the first year of research on the effects of aquatic plants on the macroinvertebrates and zooplankton of Eau Galle Lake, Wisconsin, will be presented. A discussion on the effects of *Hydrilla* on density and community composition of fishes in the Potomac River, by Killgore, Morgan, and Hurley, is included in this proceedings (see pp 236-244). Studies on the effects of aquatic plants on physical and chemical characteristics of water and sediments are reported by Barko and associates (see pp 199-224).

STUDY AREA

Sampling for vegetation and benthic macroinvertebrates was conducted at Eau Galle Reservoir, located about 80 km east of St. Paul, Minn. Construction of the dam began 9 July 1965, and final filling of the reservoir was completed in April 1969. The surface area of the reservoir is 0.6 sq km, with a maximum and mean depth of 9.0 m and 3.2 m, respectively (Kennedy 1985). The system is seasonally dimictic and eutrophic, with moderately hardwater (Barko et al. 1984, Kennedy 1985). The dominant aquatic macrophytes, *Ceratophyllum demersum* and *Potamogeton pectinatus*, contributed 58 percent and 36 percent respectively, of the standing crop between April and November 1981. Minor species include *P. nodosus*, *P. foliosus*, and *Najas flexilis* (Filbin and Barko 1985).

Study sites were located in the eastern and western sections of the lake, in water less than 1 m deep (Figure 1). At the eastern site, benthic samples were taken in three habitat types: (a) no vegetation, (b) dense stands of *Potamogeton*, and (c) dense stands of *Ceratophyllum*. Periphyton and littoral zooplankton samples were taken from the western cove of the lake, and limnetic zooplankton samples were taken from the center of the reservoir (Figure 1.)

METHODS

Macroinvertebrate samples were collected from a boat on 5 and 6 August 1986 using a hand-held benthic corer (Miller and Bingham 1987). This device is an alternative to benthic grabs or corers, which are usually deployed from a boat in a haphazard fashion. With this corer it was possible to obtain a sample in an exact location, and to ensure that plant fragments in the water column were separated from the substrate. The substrate sample was extruded from the barrel of the corer and sectioned into 5-cm increments, washed through a 0.5-mm screen
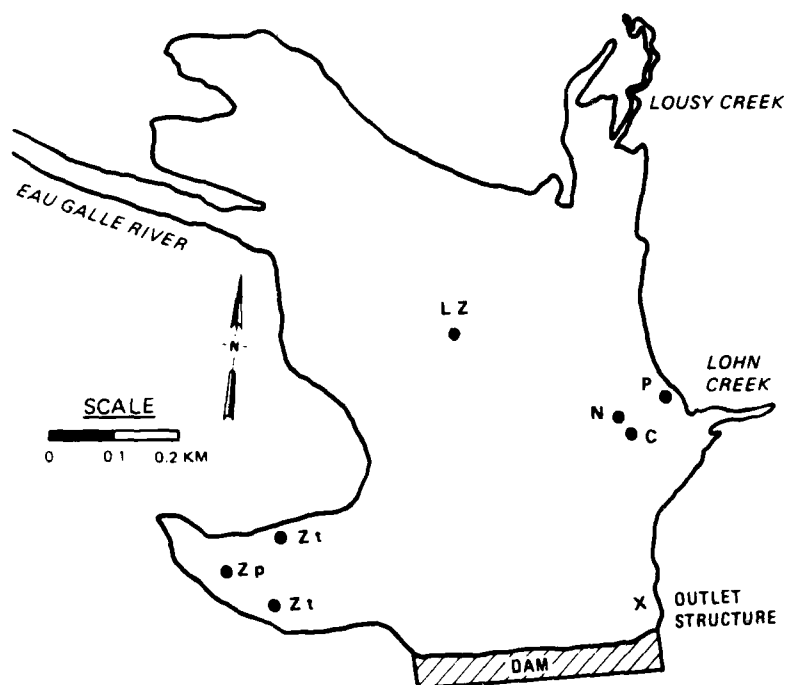
Figure 1. Map of Eau Galle Reservoir, Wisconsin, depicting locations of sampling stations (Zp - zooplankton, Zt - zooplankton traps; N - no plants; C - *Ceratophyllum*; P - *Potomageton*)

and preserved with formalin. Organisms were then stained with rose bengal to facilitate picking and were sorted in the laboratory with a dissecting microscope.

A total of 15 core samples for macroinvertebrates were collected from the substrates of dense *Potamogeton* beds (collections made at three stands of vegetation with five samples taken per site). Similarly, a total of 15 samples were collected from dense *Ceratophyllum* beds (three sites with five samples per site). Small no-plant sites were located amid the dense *Ceratophyllum* beds. Since the objective of this portion of the study was to compare the infauna in the three habitat types, care was taken in the placement of the sampler so no parts of the macrophytes were included in the samples. Each core sample was sectioned into 5-cm increments (0 to 5 cm, 5 to 10 cm, and 10 to 15 cm) for analysis.

At each area where invertebrate samples were collected, three samples were collected for total organic content (loss on ignition) and three for total particulate organic matter. For each analysis, sediments were sectioned into 5-cm increments. Total particulate content of the sediment samples was accomplished by filtering sediments through a 0.55-mm screen and drying and weighing the larger material, which consisted mainly of roots, stems, and leaves.

Zooplankton associated with aquatic plants were collected using a set of funnel traps mounted on a 0.25-sq m Plexiglas sheet (Figure 2). Each of the 25 funnels and bottles was filled with lake water prior to use. Zooplankton that migrated up in the water column entered the inverted funnel and became trapped in the bottle. Two traps were placed over dense vegetation, and two over sparse vege
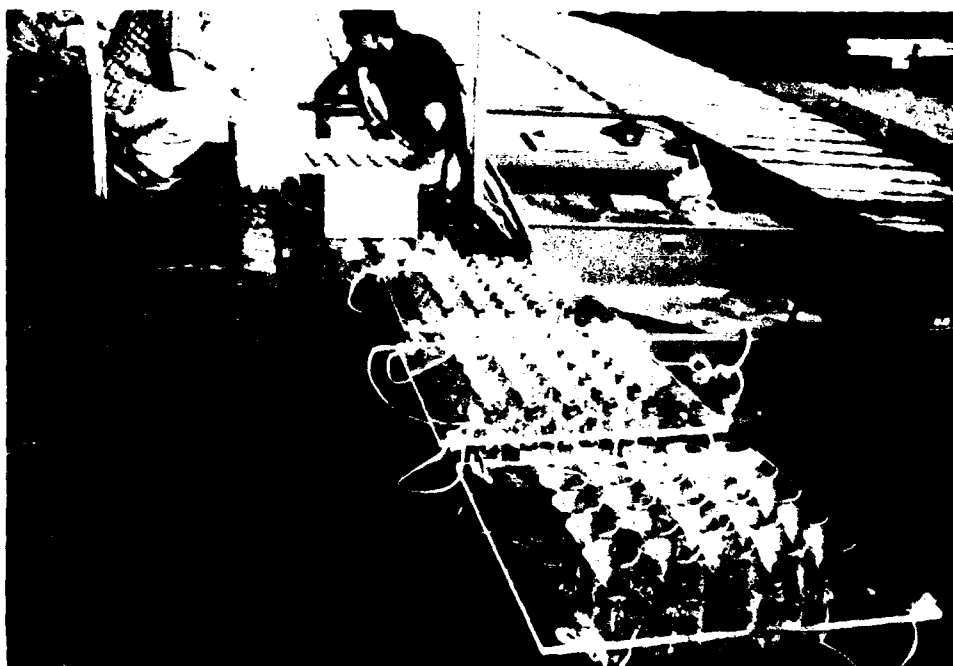
Figure 2. Zooplankton traps used at Eau Galle Reservoir, Wisconsin, 1986

tation. These traps were left in place for approximately 10 hr during the day; a second set was deployed for 10 hr during the night on 6-7 August 1986.

Pump samples for zooplankton were collected using an electric pump powered by a gasoline engine. The end of a 7.5-cm-diameter hose was placed 50 cm below the water surface for sampling in both dense and sparse weed beds. The pump was run for about 15 sec until 20 ℓ of water were collected in a carboy; this sample was concentrated by pouring it through a 153-μ zooplankton net. Samples were collected from the dense and sparse sites in *Ceratophyllum* beds once every 4 hr, starting at 12 noon on 6 and 7 August 1986.

A pair of zooplankton nets (153 and 63 μ) were used to make vertical hauls at two limnetic sites every other week starting in June 1986. All zooplankton samples were preserved in 5-percent formalin. In the laboratory, sample aliquots obtained using a Hensen-Stempel pipette were placed in either a zooplankton counting wheel (153-μ net hauls) or a Sedgewick-Rafter chamber (63-μ net hauls) for identification and counting. In the case of the trap and pump samples, the entire sample was enumerated. All species were identified at 400 diameters using the taxonomic keys found in Brooks (1957), Voight (1957), and Edmondson (1959).

RESULTS

Macroinvertebrates

The infaunal invertebrate communities were outnumbered by oligochaete worms in all three of the habitat types (no plants, dense *Potamogeton*, and dense *Ceratophyllum*). Gastropods and chironomid larvae, respectively, were the next

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most common groups in the substrates in both the no-plant and *Potamogeton* habitats. While gastropods were also the second most common invertebrate group in the bottom substrates in the *Ceratophyllum* beds, the third most common invertebrate in this habitat was the caddisfly *Leptocerus americanus*.

Invertebrate densities were approximately equal in the sites with *Potamogeton* and *Ceratophyllum* (Figure 3). However, the mean density of organisms collected from sites with no plants was about 8 percent of that at sites where there was dense vegetation. Approximately 95 percent of the benthic invertebrates from sites with aquatic plants were in the upper 5 cm of the substrate (Figure 3).

With all depth fractions pooled, the total particulate organic matter in sediments from the dense *Potamogeton* site was significantly greater than the other two areas sampled (Table 1). At all sites a trend of decreasing quantity of filterable particulate matter was noted in the lowermost depth fraction. Total organic content (loss on ignition) exhibited decreasing values at lower depths in the no-plant zone and the *Ceratophyllum* sites, but not in the sediments with *Potamogeton*.

Zooplankton

Bimonthly zooplankton hauls with the 153-μ mesh net from the limnetic stations at Eau Galle Reservoir were dominated by crustaceans (copepods and cladocerans) with relatively few numbers of rotifers (Figure 4). *Cyclops bicuspidatus thomasi*
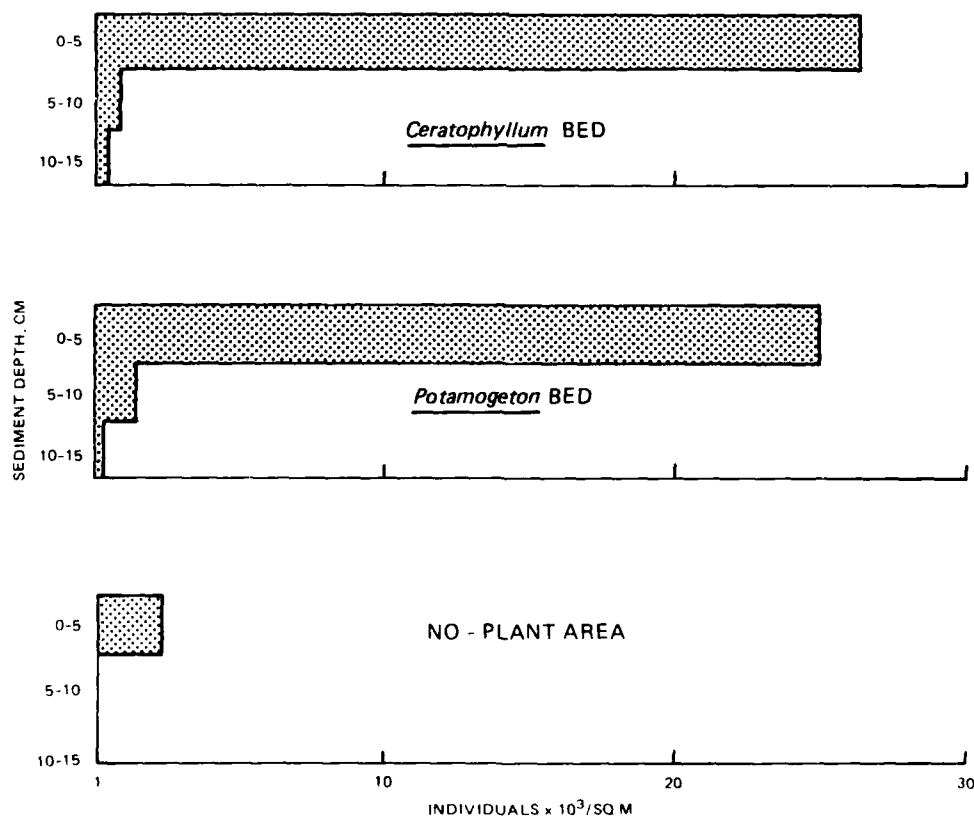

Figure 3. Total density of benthic macroinvertebrates collected from dense *Ceratophyllum* and *Potamogeton* beds and a no-plant zone, Eau Galle Reservoir, August 1986

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Parameter	Ceratophyllum		Potamogeton		No Plants	
	T Organic	P Organic	T Organic	P Organic	T Organic	P Organic
Depth						
0-5	3.6	421.5	4.7	321.0	5.8	1,720.1
5-10	3.7	240.5	6.3	112.1	4.4	654.7
10-15	2.9	179.5	5.6	63.0	3.5	329.1
Number	8	9	8	9	8	9
Average	3.4[y]	280.5[a]	4.4[x]	901.3[b]	5.5[xy]	165.4[a]
Standard deviation	0.7	125.8	1.9	978.9	1.5	127.8

Note: Comparisons are made among plant types for total organic (T) and total particulate (P) organic content using Duncan's multiple range test. Means with the same superscript are not different at the 0.05 level.

LEGEND
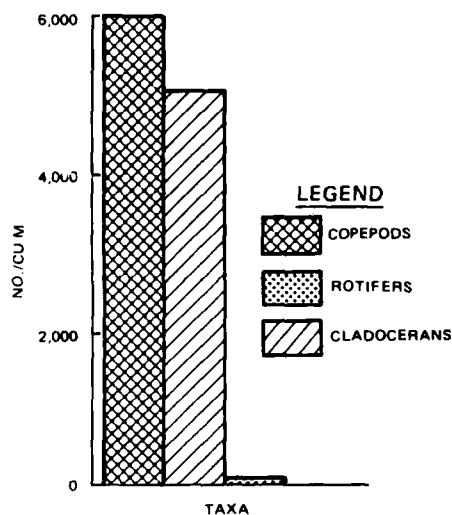
COPEPODS

ROTIFERS

CLADOCERANS

Figure 4. Zooplankton community at two limnetic sites, Eau Galle Reservoir, Wisconsin

comprised about 60 percent of the mature copepods, and *Daphnia retocurva* made up 70 percent of the cladoceran community. In the hauls taken with the 64-μ mesh net, rotifers numbered about the same as the mature crustaceans, with *Keratella cochlearis* and *Keratella quadrata* dominating this portion of the community.

The zooplankton traps captured organisms which crawl about on plants and are usually not free in the water column. *Ceriodaphnia* sp. chydorids (cladocerans) and ostracods, which are relatively small crustaceans, comprised the majority of the zooplankton community associated with submersed vegetation. The traps in dense vegetation were dominated by copepods with relatively few numbers of ostracods, rotifers, and cladocerans. The traps in sparse vegetation collected about 40 percent fewer individuals (Figure 5).

The pump samples collected a slightly different zooplankton community than the traps or nets. Ostracods were more abundant in the trap samples than the pump samples (Figures 5 and 6). Like the trap samples, the rotifers were more
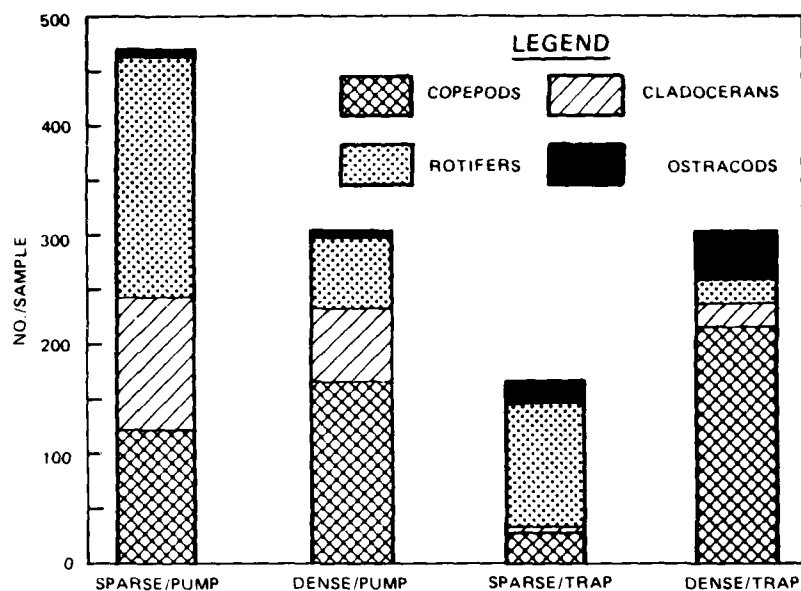
Figure 5. (Comparison of zooplankton communities in sparse and dense vegetation determined from samples collected by pumping 20 of water and by using zooplankton traps, Eau Galle Reservoir, August 1986
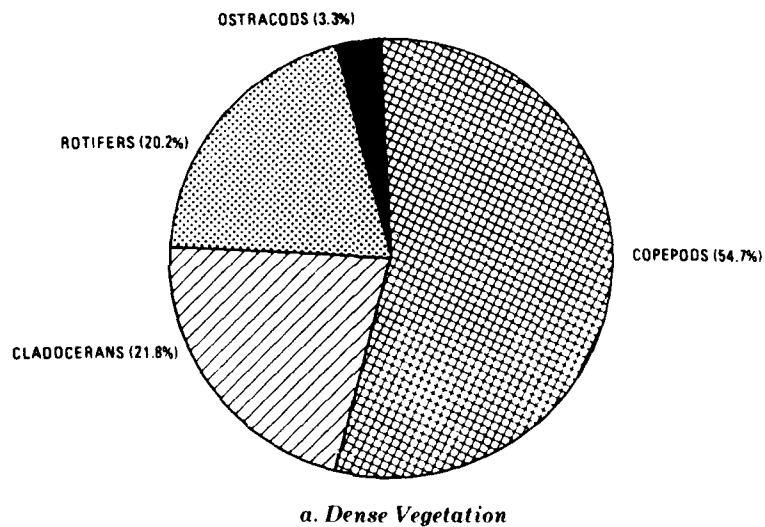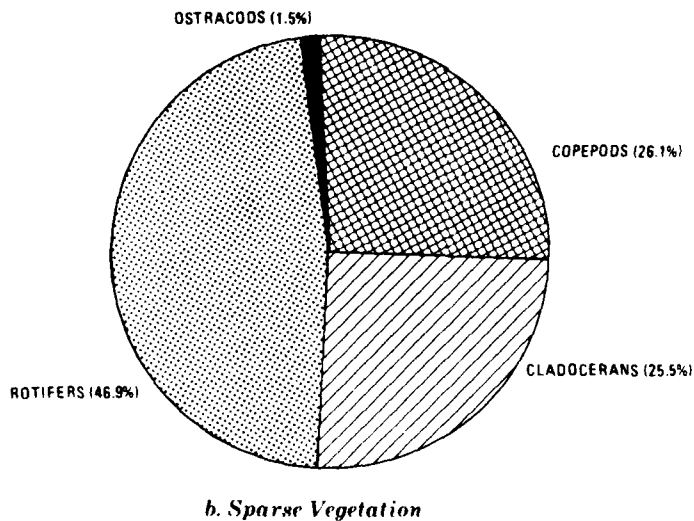

a. Dense Vegetation

Figure 6. Zooplankton community composition from areas with dense and sparse *Ceratophyllum*, determined with samples collected by pumping 20 of water, Eau Galle Reservoir, August 1986

b. Sparse Vegetation

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abundant in sparse vegetation than in dense. Densities of copepods and rotifers were dissimilar. In the sparse vegetation, rotifers made up 46.9 percent of the community, with equal numbers of copepods (26.1 percent) and cladocerans (25.5 percent). However, in dense vegetation, copepods comprised more than 50 percent of the sample, while rotifers (20.2 percent) and cladocerans (21.8 percent) were subdominant.

DISCUSSION

Macroinvertebrates

Numerous studies have shown the value of stems and leaves of aquatic plants for macroinvertebrates (Ball and Hayne 1952, Gerking 1962, Pennak 1971, Nichols 1974, Killgore 1979, Keast 1984, Minshall 1984). However, this study shows that presence of submersed vegetation also enhances invertebrate densities in the substrates of the plant beds. The data from Eau Galle Reservoir demonstrated that vegetated versus nonvegetated sites only a few meters apart differed dramatically with respect to invertebrate densities. Other workers have reported similar findings. Menzie (1980) determined that biomass of chironomids in the sediments of a plant-filled cove to be about 16 times that of neighboring nonvegetated areas. Watkins, Shireman, and Haller (1983) reported that the infaunal invertebrate numbers in the sediment of a *Hydrilla* bed were approximately four times the numbers present in the sediments of nearby nonvegetated areas.

Brouha and von Geldern (1979) suggested that the diversity of benthic invertebrates was greater at vegetated sites because of sediment stability and the presence of organic matter as a source of food. At Eau Galle Reservoir, sediments from the no-plant zone did not exhibit significantly less organic matter than the sediments from the areas with plants (Table 1). Particulate organic matter was significantly greater in the sediments with *Potamogeton*, although similar in the no-plant zone and areas with *Ceratophyllum*. Total organic content and particulate organic matter among these sites are not as strikingly different as the macroinvertebrate density data, and do not appear to be causative factors.

Olsson (1981), in a study of a Swedish River, demonstrated that the mechanical stress of ice in the sediments was reduced by the presence of aquatic vegetation. He found that macroinvertebrates survived freezing for several months when aquatic plants were in the sediments. The littoral region at Eau Galle freezes during the winter, and this could affect invertebrate distribution. In a study at Lake Washington, Chen and Barko (pp 205-214 of this proceedings) reported that sediments became reduced (i.e., anoxic) below 30 mm, which would stress the majority of invertebrate taxa. Additional studies on macroinvertebrate dis tri-bution in vegetated and nonvegetated sites will be conducted as part of this research program.

Zooplankton

Cladocerans are relatively large and slow moving; hence, they are readily preyed upon by fishes. Rotifers are small, slow moving, and not a preferred food item.

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Although copepods are small, they move rapidly and are not as likely to be preyed upon by fish. At Eau Galle Reservoir, when densities of rotifers were high, copepods were low, and vice versa. In areas with sparse vegetation, rotifers were more numerous than copepods, suggesting that fishes visually selected and captured the copepods. In the dense vegetation, the faster moving copepods were probably better able to evade predation from small predatory fishes than in sparse vegetation. Also, since the rotifers and cladocerans are distributed more by current than are copepods, advection of these organisms into the weedy areas is reduced. This fact, coupled with the high probability of predation from the larger copepod populations in these areas, could explain reduced numbers of these plankters in this microhabitat.

FUTURE CONSIDERATIONS

During preliminary planning for this project, nine tasks were identified for study (Table 2). This paper has provided preliminary results of tasks IV and IX; Killgore and Morgan (these proceedings) present information on Tasks I and II. During the summer of 1987, additional work on zooplankton, benthic macroinvertebrates, attached invertebrates, and larval and adult fishes will be initiated.

Table 2
Subtasks for Research on the Habitat Value of Aquatic Plants

I. Tropic associations in aquatic plant beds
    a. Standing crop of fishes
    b. Stomach analyses of selected fishes
    c. Benthic invertebrate distribution
    d. Attached invertebrate analysis
    e. DO, pH, and water temperature at different depths and differences from shore
II. Diurnal/seasonal variations in fish distribution in aquatic plant beds in the Potomac River
III. Selected removal of aquatic plants for management
IV. Abundance and distribution of invertebrates (zooplankton and macroinvertebrates) in aquatic plant beds (Eau Galle Reservoir)
    a. Analysis of density and spatial distribution of zooplankton and attached invertebrates on aquatic vegetation with various types of structure
    b. Evaluation of DO, pH, and water temperature in the water column and relate to macroinvertebrate distribution
V. Periphyton colonization on aquatic plants
VI. Invertebrate population demographics in relation to distribution of aquatic plants
VII. Benthic invertebrate density and community composition in aquatic plant beds
VIII. Use of aquatic vegetation by fish larvae
IX. Evaluation of depth distribution of invertebrates in sediments with and without aquatic vegetation

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Distribution and Abundance of Fishes in Aquatic Vegetation

by
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INTRODUCTION

Submersed aquatic vegetation mediates both fish distribution and abundance by creating structurally complex habitats (Crowder and Cooper 1979) that provide food and shelter (Barnett and Schneider 1974, Moxley and Langford 1982). Fish abundance can be substantially higher in vegetated habitats (Laughlin and Werner 1980; Holland and Huston 1984) while optimal foraging conditions usually occur in areas with intermediate plant densities (Reynolds and Babb 1978; Savino and Stein 1982; Durocher, Provine, and Kraai 1984; Wiley et al. 1984). Although aquatic vegetation is an important component of aquatic ecosystems, its consideration in fishery management programs is often secondary to the control efforts employed to minimize its impact to primary water uses such as recreational boating. However, many exotic plants, such as *Hydrilla verticillata*, can increase their coverage to a point where only a small percentage can be economically controlled. The remaining uncontrolled areas undoubtedly have both indirect and direct benefits to fish productivity, but limited quantitative data are available to substantiate their value. If aquatic plant management programs consider the value of all water uses when developing control strategies, including the fishery resource, the influence of aquatic plants on the distribution and abundance of fishes must be documented.

The recent introduction of *Hydrilla* in the Potomac River is an example of the establishment and subsequent rapid spread of an exotic macrophyte. In recent times, this river was devoid of any substantial amounts of instream structure such as aquatic vegetation. In 1981, *Hydrilla* was identified in the Potomac River and spread to approximately 1,100 ha by 1986 (Rybicki et al., in preparation). Only 1 percent of the aquatic vegetation in the Potomac River was controlled in 1986 using a mechanical harvester,†† leaving vast areas available for fish utilization. Based on the substantial increase of *Hydrilla*, this study was initiated to assess the influence of aquatic vegetation on fishes in the Potomac River as part of an overall effort to document its habitat value. Our objectives were to determine the seasonal and diurnal distribution of fishes in different densities of aquatic vegetation and to conduct a preliminary study on techniques that could be used to quantify the standing crop of fishes in aquatic vegetation.

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DESCRIPTION OF STUDY AREA

The study area was located in the tidal Potomac River between Woodrow Wilson Bridge (166 km) and Gunston Cove (140 km) near Alexandria, Va. The Potomac River has semidiurnal tides, an average depth of 5.8 m with a deep channel (>8 m), and adjacent wide shallow shelves (Environmental Laboratory 1985). Most aquatic plants are established on the shelves in 1 to 3 m of water and decline along the side channel. Plants begin to emerge from the substrate in May and reach their peak biomass in August. Senescence occurs for most plants from November to December, and they frequently form large floating mats, some in excess of 2 ha, in the main channel.

Common plant species in the study area included *H. verticillata*, *Myriophyllum spicatum*, *Vallisneria americana*, *Ceratophyllum demersum*, and *Heteranthera dubia*. Except for *Hydrilla*, the aquatic vegetation had a patchy distribution with two or more species co-dominating. *Hydrilla* forms an impenetrable mat during the summer and fall months and is the dominant species where it occurs.

METHODS

Seasonal and diurnal fish collections were made in three different densities of aquatic vegetation using a boat-mounted electroshocker. Seasonal collections reflected the habitat conditions created by the annual life cycle of these plants: May (plant emergence), August (peak biomass), and November (senescence). Diel samples (dawn, midday, and night) were made to account for the 24-hr variation in fish abundance. Prior to the fish collections in May, August, and November, study sites were identified that represented three relative levels of aquatic vegetation density: no plants, intermediate plant density, and high plant density. Five replicate samples were collected with the electroshocker in areas with similar plant density at each site on each sampling date. One replicate consisted of 5 min of continuous shocking.

All fish collected were measured to the nearest millimetre and weighed to the nearest 0.1 g. Standard length was used for all species with heterocercal tails, while total length was used for species with homocercal tails. Replicate water quality (temperature, dissolved oxygen, pH, conductivity, and turbidity) measurements were made during each sampling event. Ten plant biomass samples were collected at each site in May, August, and November. Each sample was separated by species, and wet and ash-free dry weights were determined. A two-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (SAS Institute, Inc. 1985) were used to determine and identify significant differences in fish abundance (number of fish captured per 5 min of shocking) between sites and diel sampling periods monthly.

A preliminary evaluation using modified pop nets as originally described by Larson, Johnson, and Lynch (1986) was made to determine the standing crop of fishes in aquatic vegetation. The nets were constructed with 5-mm nylon mesh and were rectangular shaped with a width of 3.05 m, a length of 3.05 m, and a depth of 2.8 m. The bottom was open in order to place the net over the aquatic

vegetation. The top of the net was attached to a float line made of 2.5-cm-diam PVC; and the bottom of the net was attached to 1.3-cm-diam PVC with "ray-bar" inserted for weight. Divers placed the net over the aquatic vegetation and securely anchored the bottom line to the substrate.

In high-density vegetation, the divers removed a small portion of the aquatic vegetation under the bottom line in order to keep it flush with the substrate. The divers then attached the float line to the botom line using two large U-bolts with a 15-m-long rope attached to each bolt. The nets were left unattended for approximately 3 hr to allow fishes to naturally disperse throughout the aquatic vegetation bed. After this period, the float lines were released by pulling the U-bolts free, and the nets rose to the surface. The majority of vegetation was removed from each net, and the fishes were collected using a seine. Usually, five seine hauls were necessary to remove all fishes from the net.

Six pop nets were placed in aquatic vegetation. Three nets were placed parallel to the shoreline in high vegetation density (approximately 1,000 g/m² dry weight), primarily *Hydrilla*. Three additional nets were placed perpendicular to the shoreline in intermediate vegetation density (approximately 400 g/m² dry weight), composed of patchy distributions of *Myriophyllum* and *Heteranthera*. All nets were set in the morning and tripped in midafternoon. Collected fishes were identified, counted, and measured to the nearest millimetre.

RESULTS AND DISCUSSION

Plant biomass varied considerably between sites. Plant biomass in the high plant density (HPD) site was only 33 g/m² dry weight in May but increased to over 1,000 g/m² in August. Plant biomass in the intermediate plant density (IPD) site was 30 to 60 percent less than the HPD site. *Myriophyllum* was the dominant species in the HPD site in May with a "topped out," patchy distribution. *Hydrilla* was beginning to emerge from the substrate in May and was the dominant species at the IPD site at this time. *Hydrilla* was the dominant macrophyte in the HPD site during August and November when plant density was highest, whereas the IPD site was comprised of several codominating species with a patchy distribution. No measurable amount of aquatic vegetation was observed at the no plant (NP) site throughout the season. During November, senescence was occurring, and most plants were floating on the water surface.

A total of 3,883 fishes comprising 30 species were collected by electroshocking during the study. Eleven species accounted for 95 percent of the total individuals collected, which included pumpkinseeds (*Lepomis gibbosus*), white perch (*Morone americana*), bay anchovy (*Anchoa mitchilli*), inland silverside (*Menidia beryllina*), largemouth bass (*Micropterus salmoides*), yellow perch (*Perca flavescens*), alewife (*Alosa pseudoharengus*), menhaden (*Brevoorita tyrannus*), spottail shiner (*Notropis hudsonius*), banded killifish (*Fundulus diaphanus*), blueback herring (*Alosa aestivalis*), brown bullhead (*Ictalurus nebulosus*), gizzard shad (*Dorosoma cepedianum*), golden shiner (*Notemigonus crysoleucas*), bluegill (*Lepomis macrochirus*), and Atlantic silverside (*Menidia menidia*).

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Except for the IPD site in May, more species were collected in vegetated areas (18 to 23) than in areas devoid of vegetation (9 to 13). Low species richness at the IPD site in May (seven species) could be due to a relatively low dissolved oxygen (5.3 ± 0.6 mg/l) measured only at this site and a low preference by fishes to emerging *Hydrilla* (which was the dominant plant at the IPD site in May) as opposed to "topped out" stand of *Myriophyllum*, the only significant structure during the late winter and early spring months.

Fishes tend to associate with overwintering vegetation for food and cover and disperse to the more established macrophyte beds in the summer and fall (Hall and Werner 1977). Therefore, overwintering *Myriophyllum* may be important to long-term survival of fishes in the Potomac River. The majority of fishes captured in the NP site were pelagic (menhaden, blueback herring, Atlantic silverside, bay anchovy), whereas cover-oriented fish, such as pumpkinseed, largemouth bass, yellow perch, and brown bullheads, were associated with vegetated areas. For example, over 200 largemouth bass were collected in the vegetated areas, while this species was never collected at the NP site. Aquatic plants are obviously important to sport fish production in the Potomac River, especially if optimal plant densities can be determined and used to establish control objectives (Wiley et al. 1984).

Aquatic plants influenced the seasonal and diurnal distribution of fishes in the Potomac River. The ANOVA showed a significant difference in fish abundance between sites and time of day during all three sampling months (Table 1). No significant interaction between plant density and collection time was evident. Except for during May, all three sites were significantly different from each other in terms of fish abundance, with the highest abundance consistently occurring at the IPD site followed by the HPD and NP sites (Table 2). In May, fish abundance was relatively low at the IPD site, while a relatively high number of fishes were collected at the NP site, primarily due to the occurrence of pelagic saltwater species (alewife, menhaden, and anchovies) entering fresh water to spawn. In August and November, 3 to 11 times more fishes were collected in vegetated areas than in

Table 1

Two Factor ANOVA Comparing Mean Number of Fishes Captured per 5-min Shocking Period (n = 5)
Between Three Sites Having Different Densities of Aquatic Plants
and During Three 24-hr Collection Periods

Source of Variation	d.f.	SS	F	P
May 1986				
Density of plants	2	2,286.2	5.87	0.006
Time of day	2	2,659.4	6.83	0.003
Density* time	4	1,518.7	1.95	0.123
August 1986				
Density of plants	2	22,144.0	11.96	0.001
Time of day	2	8,188.6	4.42	0.019
Density* time	4	3,895.0	1.05	0.394
November 1986				
Density of plants	2	13,328.5	58.53	0.001
Time of day	2	2,843.7	12.49	0.001
Density* time	4	1,067.7	2.34	0.073

Table 2
Comparison of Mean Number of Fish Captured per 5-min Shocking Period (n = 5)
Between Time of Day and Density of Vegetation
Using Duncan's Multiple Range Test

Source of Variation	Mean ± 1 SD		
May 1986 Density of plants	No Plants	High	Intermediate
	16.6 ± 19.2	5.1 ± 20.6	0.8 ± 2.1
Time of day	Dawn	Afternoon	Night
	2.8 ± 4.1	8.5 ± 18.3	21.2 ± 20.5
August Density of plants	No Plants	High	Intermediate
	23.3 ± 32.9	49.9 ± 18.3	77.7 ± 42.6
Time of day	Dawn	Afternoon	Night
	45.9 ± 38.5	36.4 ± 21.2	68.6 ± 48.0
November 1986 Density of plants	No Plants	High	Intermediate
	3.8 ± 4.3	26.1 ± 14.1	45.9 ± 18.8
Time of day	Dawn	Afternoon	Night
	17.5 ± 17.6	22.1 ± 19.1	36.2 ± 25.3

* Note: Values not underlined are significantly different at p = 0.05.

areas without plants. In a similar study, Holland and Huston (1984) reported that northern pike (*Esox lucius*) abundance was more than 10 times greater in vegetated areas than in an area with no vegetation. Comparison of means also showed that fish abundance was highest at night, while there was no significant difference between the morning and afternoon collections.

These data indicate that studies designed to quantify maximum fish biomass in vegetated areas should be conducted during the night and that fish abundance is highest in areas with intermediate plant density. This corroborates other studies that have shown that optimal fish productivity occurs at intermediate levels of structural complexity (Crowder and Cooper 1979; Colle and Shireman 1980; Savino and Stein 1982; Wiley et al. 1984; Durocher, Provine and Kraai 1984). Profuse growth of *Hydrilla* can occupy the entire water column, which can decrease foraging efficiency of larger predators as well as restrict the movement of smaller fishes. However, certain species of fishes, such as the banded killifish and freshwater eel (*Anguilla rostrata*), seem to tolerate dense plant growth. The banded killifish was commonly observed swimming near the water surface directly over the plants, while the freshwater eel was found along the bottom where plant biomass is reduced. For these and other species, a higher productivity rate may occur when large areas of dense aquatic plants dominate littoral zones while larger predators are confined to the periphery of the plant beds. Although this study showed that intermediate densities of aquatic vegetation contained more species and numbers of fishes than areas having dense growths, it should be recognized that the inefficiency of collecting stunned fish in dense *Hydrilla* beds using a dip net may bias the results and contribute to an underestimate of fish abundance in these areas.

Given the numerous problems associated with collecting fishes in areas with dense aquatic plants, nonconventional techniques must be developed to obtain

unbiased estimates of fish abundance in aquatic plants. The pop nets used in this study appear to accommodate these criteria and can provide reliable fish standing crop estimates in virtually any density of aquatic plants (Table 3). Our preliminary data, which were based on only three replicate samples, showed that the HPD site, consisting of *Hydrilla* (approximately 1,000 g/m^2 dry weight), had 16.7 ± 5.5 fish per 9.3 m^2 (17,949 fish/ha). In contrast, the IPD site, consisting of *Myriophyllum*, *Vallisneria*, and *Heteranthera* (approximately 400 g/m^2 dry weight), had 91.3 ± 76.0 fish per 9.3 m^2 (97,813 fish/ha).

Other studies have also observed a high number of fishes residing in aquatic vegetation. Shireman, Colle, and DuRant (1981) and Haller, Shireman, and Durant (1980) reported 13,000 to 205,000 fish/ha in vegetated areas in Orange Lake, Florida, while Barnett and Schneider (1974) estimated 86,000 to 2.5 million fish/ha in several locations in central Florida.

Although these estimates are quite variable due to sampling techniques, density of the vegetation, and patchy fish distribution, they exemplify the importance of aquatic vegetation to fish productivity. The pop net data clearly show that intermediate densities of aquatic vegetation support more fish per unit area than high plant density. Differences in abundance between nets at the intermediate density site were probably related to their placement. The net placed closest to the shoreline had the lowest number of fish while the net situated near the "edge" of the plant bed had six times more fish. These data suggest that fish segregate in areas with similar density of plants and prefer the "edge" of large aquatic plant beds in order to utilize both pelagic and structurally complex areas for foraging and predator avoidance. Furthermore, diurnal fluctuations in pH may also influence fish distribution. Rybicki et al. (in preparation) found that pH shifts from 7.0 near the edge of the plant bed to 10.0 near the shoreline during the afternoon. Fish may be avoiding these drastic increases in pH by congregating near the edge where the pH is neutral.

The pop nets can provide valuable data on fish abundance and distribution in aquatic vegetation. Larson, Johnson, and Lynch (1986) reported that the efficiency

Table 3
Number of Fishes and Species Captured in Intermediate
and High Vegetation Density Using Pop Nets*

Density of Vegetation	Number of Fishes	Number of Species
High density		
Pop net 1	14	4
Pop net 2	13	4
Pop net 3	23	7
Mean ± 1 SD	16.7 ± 5.5	5.0 ± 1.7
Estimated number hectare	17,949	--
Intermediate density		
Pop net 1	178	7
Pop net 2	60	6
Pop net 3	36	5
Mean ±1 SD	91.3 ± 76.0	6.0 ± 1.0
Estimated number hectare	97,813	--

* Each net sampled 9.3 m^2 of submersed aquatic vegetation

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of the pop nets was near 100 percent and can accurately sample the entire fish assemblage at an artificial structure. Although placement and sampling of the pop nets usually require divers, they are not as labor intensive as rotenone sampling inside large block nets and minimize equipment bias that is inherent with active capturing techniques such as an electroshocker. Furthermore, replicate numbers of pop nets can be easily deployed in aquatic vegetation to account for the high variability in fish distribution common in structurally complex habitats.

The recent introduction of aquatic vegetation in the Potomac River has provided new habitat for sport and commercial fish species. The habitat diversity created by these plants has contributed to an increase in fish productivity. However, this study showed a complex relationship between fish distribution and the physical and chemical environment in aquatic vegetation, suggesting that additional quantitative studies are required to determine rational management strategies that consider the habitat value of aquatic plants. Future studies will be directed toward predicting the standing crop of fish using the pop net technique and evaluating the trophic relationships that occur in aquatic vegetation. This information will contribute to a better understanding of how aquatic plant control activities can affect the integrity of fish community structure.

CONCLUSIONS

The following conclusions can be made on the effects of aquatic vegetation to fish in the Potomac River:

- Fish abundance was substantially higher in aquatic vegetation. Up to 11 times more fish were collected in aquatic vegetation than in areas without plants. Sportfish species such as largemouth bass, yellow perch, and sunfishes were common only to areas with aquatic vegetation, while pelagic species such as alewife, menhaden, and herring dominated the fish assemblages in areas without aquatic plants.

- Fish distribution apparently responds to seasonal shifts in plant biomass. During peak biomass periods ($>$1,000 g/m^2, dry weight), fish abundance was highest in areas with intermediate plant density (78 fish/5 min shocking), followed by high plant density (50 fish/5 min shocking) and sites with no aquatic plants (23 fish/5 min shocking). When most plants were beginning to emerge from the substrate in the spring, more fish were found in areas with high plant densities (30 g/m^2, dry weight), usually associated with "topped out" stands of *Myriophyllum*.

- Number of fish captured at night was significantly higher than the dawn or afternoon collections. Therefore, maximum fish densities occur at night in areas with intermediate plant density.

- Pop nets can provide unbiased data on standing crop of fishes in aquatic vegetation. They are less labor intensive than conventional techniques such as rotenone, and can therefore be replicated to account for the high variability in fish distribution. Almost six times more fish were collected in areas with intermediate plant density (400-500 g/m^2, dry weight), consisting of several codominate species, than in areas with high plant density ($>$1,000 g/m^2, dry weight), consisting mostly of *Hydrilla*. Results of the pop nets suggest that fish segregate in areas with similar plant density and prefer the "edge" of

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the plant bed (178 fish/9.3 m²) rather than near the shoreline (36 fish/9.3 m²) in order to utilize both pelagic and vegetated habitats and avoid diurnal shifts in pH and dissolved oxygen.

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Effects of Temperature and Sediment Type
On Growth and Morphology of
Monoecious and Dioecious *Hydrilla*

by

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INTRODUCTION

Hydrilla verticillata (L.f.) Royle (Family Hydrocharitaceae) is a genetically variable, polymorphic, submersed macrophyte (Caspary 1858, Scannell and Webb 1976, Cook and Luond 1982, Verkleij et al. 1983). Native to southeast Asia and Australia (Cook and Luond 1982; Swarbrick, Finlayson, and Cauldwell 1982), *Hydrilla* has an extremely wide and expanding geographical range. Its distribution, though somewhat disjointed, reaches all continents except mainland South America where it has not yet been documented (Cook and Luond 1982). Often gregarious in nature, *Hydrilla* can grow in a variety of water quality conditions, e.g., from clear to turbid, strongly alkaline to acidic, fresh to brackish, and in oligotrophic to eutrophic systems (Burkhalter et al. 1978, Verkleij et al. 1983). Genetic varieties or 'biotypes' of this species are classified sexually as monoecious, bearing both male and female flowers on the same plant, or dioecious, bearing either male or female flowers on separate plants (Ernst-Schwartzenbach 1945, Cook and Luond 1982). Although the absolute number of *H. verticillata* biotypes is unknown, the literature indicates that there are many scattered throughout trophical and subtropical regions of the world that could become established in North America (Cook and Luond 1982).

Hydrilla found in the United States apparently resulted from two separate introductions. The female dioecious biotype, initially sited in Florida about 1960 (Blackburn et al. 1969) has become one of the most prolific and troublesome aquatic plants in southern states (Haller 1976). Infestations of *Hydrilla* interfere with water flow and cause numerous navigational and recreational use problems. Dioecious *Hydrilla* occurs throughout the southeast — most notably in Florida — up the east coast to South Carolina and as far west as California. A more recently discovered monoecious biotype was reported in 1982 in the Potomac River near Washington, DC (Steward et al. 1984). In 4 years, the plant has spread to at least five states in the northeast and is rapidly colonizing downstream areas of the Potomac River (Environmental Laboratory 1985). Based on isoenzyme banding pattern and chromosome number, Verkleij et al. (1983) confirmed that the two

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strains are indeed genetically distinct. They further hypothesized that the genetic variation of *Hydrilla* biotypes may indicate differences in survival strategies, and possibly differences in response to control techniques.

The severity of aquatic plant management problems associated with dioecious *Hydrilla* has prompted numerous studies into the ecology of this biotype. As a rooted aquatic plant, it is able to derive nutrients from pools in the sediment as well as from the overlying water column (Barko and Smart 1980, Barko 1982, Steward 1984). Its competitive success is primarily attributed to its efficient modes of vegetative reproduction, i.e., fragmentation and the production of tubers, turions, rhizomes, and stolons (Haller 1976; Steward and Van 1984), and its low light saturation and compensation points for photosynthesis (Van, Haller, and Gerrard 1976). Furthermore, its phenomenal elongation capability allows it to concentrate light-receptive structures at or near the water surface, forming a foliar canopy that essentially shades out many native competitor species (Barko and Smart 1981a).

The interactive and independent influences of a variety of environmental parameters on the growth of submersed macrophytes, including dioecious *Hydrilla*, have been extensively studied by the Waterways Experiment Station (WES), Environmental Laboratory. Among these factors, the effects of water temperature and sediment composition have received considerable attention. In investigations of Barko and Smart (1980; 1981a, b; 1983) and Barko, Hardin, and Matthews (1982), macrophyte species frequently exhibited similar growth patterns in response to changes in the aforementioned variables; however, the range or magnitude of response varied according to species. Information to date indicates submersed macrophytes are tolerant of a broad range of temperatures and that total biomass production generally increases with increased temperature to at least 28° C. Specific differences in lower temperature tolerance and life history could consequently affect the latitudinal expanse of certain species. Sediment composition (i.e., texture and organic matter content) also has a pronounced influence on macrophyte growth, and could be of primary importance in the distribution of submersed plants. Recent investigations have demonstrated that growth is relatively poor on low-density organic sediments and sands as opposed to fine-textured inorganic sediments. Mechanisms of growth suppression appear to be linked principally with nutrition (Barko and Smart 1986), although inhibitory influences of organic compounds in the sediment interstitital water have been postulated as well (Barko and Smart 1983).

Laboratory determination of growth trends peculiar to each biotype is of special interest because of the potential adaptive similarities of monoecious and dioecious *Hydrilla* in the United States. Appropriate assessment of the distribution and site-specific growth potential of monoecious *Hydrilla* depends largely on understanding its specific environmental tolerances and requirements. Here we present results of an investigation designed to contrast growth of the two biotypes of *Hydrilla* over a range of temperatures on organic and inorganic sediments. Comparative variations in response to these variables could provide further insight into species plasticity and potential expansion of the distribution of *Hydrilla verticillata* in North America.

MATERIALS AND METHODS

The investigation was conducted during August and September 1985 in the Environmental Laboratory greenhouse facility at WES (Barko and Smart 1981a). Twelve 1,200-*l* white fiberglass tanks were filled with a general-purpose culture solution (Smart and Barko 1985) to a depth of ca. 83 cm. The solution is a moderately alkaline medium with a pH upon preparation of ca. 8.3. Major nutrients, e.g., N and P, that the plants could obtain from the sediments, were omitted from the solution to minimize algal growth inside the tanks. Continuous water circulation and temperature control were provided by liquid circulators that were plumbed independently to each tank. During the study, temperatures were monitored twice daily, and minor thermostat adjustments were made as necessary.

Monoecious and dioecious *Hydrilla* used in the study were obtained from 6-week-old stock cultures grown in the WES laboratory. Monoecious stocks were initially established from tubers collected from the Potomac River, Virginia. The dioecious plants were cultured from stem apexes clipped from colonies in Lake ̄· ̈·nole, Florida.

Six experimental tanks were allotted per biotype (12 total) and were ̇· ̇·iged in sets of two, corresponding with the 4° C increase in temper·'   2° to 32° C. Four 2-*l* replicates of an organic sediment from Buckho̅ı .. ̲ake. Ontario, Canada, and four 2-*l* replicates of an inorganic sediment from Brown's Lake, WES. were placed in respective tanks. Table 1 presents sediment characteristics based on analytical procedures described in Barko and Smart (1986). Sediment containers were planted separately with four 15-cm-long apical clippings of either monoecious or dioecious *Hydrilla*. Immediately after planting, the tanks were covered with a neutrally absorptive shade fabric that reduced natural diel irradiance to 51 percent.

At the end of 5 weeks, the plants were harvested, measured, oven-dried (at 80° C), and weighed as discrete components of above- and below-ground biomass. Response variables that were examined included total biomass, root-to-shoot ratio, shoot length, shoot number, tuber number, and tuber mass. Techniques for deriving shoot length and shoot number of individual plants are described in Barko and Smart (1981a). Tubers of all sizes were included in the count.

Table 1
Characterization of Sediments*

Sediment Parameter	Source	
	Brown	Brown
Texture, percent		
Fine particles (̲ 50 μ diam)	90.0 ± 0.0	80.0 ± 0.0
Coarse particles (> 50 μ diam)	10.0 ± 0.0	20.0 ± 0.0
Dry weight density, g ml⁻¹	0.76 ± 0.01	0.07 ± 0.00
Total organic matter, percent	5.6 ± 0.0	50.2 ± 0.4

* Values are means and standard errors based on duplicate or triplicate determinations.

RESULTS AND DISCUSSION

A synoptic analysis of variance (ANOVA) is presented in Table 2. These data are provided as a guide in assessing the relative significance of independent and interactive effects of temperature and sediment on the growth and morphological responses of monoecious and dioecious biotypes, as discussed below.

Table 2
Synoptic Two-Way ANOVA of Growth Differences in Dioecious and Monoecious
Hydrilla Relative to Temperature and Sediment

Response Variable	Environmental Variable*	Dioecious		Monoecious	
		P	F	P	F Value
Total biomass	Temp	<0.001	165	<0.001	181
	Sed	<0.001	216	<0.001	348
	Temp × Sed	<0.001	31	<0.001	50
Root:shoot	Temp	<0.001	29	<0.001	22
	Sed	NS	<1	<0.001	13
	Temp × Sed	<0.05	3	<0.001	7
Shoot length	Temp	<0.001	1606	<0.001	728
	Sed	<0.001	1299	<0.001	178
	Temp × Sed	<0.001	138	<0.001	16
Shoot number	Temp	<0.001	80	<0.001	71
	Sed	<0.001	15	<0.001	89
	Temp × Sed	<0.01	4	<0.001	11
Tuber number	Temp	<0.05	3	<0.001	12
	Sed	<0.05	6	<0.001	17
	Temp × Sed	NS	2	<0.01	4
Tuber mass	Temp	NS	1	<0.001	7
	Sed	NS	<1	<0.001	29
	Temp × Sed	NS	<1	<0.05	3

* Temp = temperature. Sed = sediment. Temp × Sed = interaction of temperature and sediment.

Total biomass and root-to-shoot ratio

Biomass production in monoecious and dioecious *Hydrilla* was severely inhibited at 16° C and below (Figure 1). Above 16° C, growth increased up to 28° C with the thermal optima for both biotypes occurring within the range of 28° to 32° C. As anticipated, the plants were far less responsive to the organic sediment than the inorganic sediment. particularly from 20° to 32° C where sediment type had a consistently significant effect on biomass accrual. Overall differences in biomass due to biotype were minor.

Unlike the trends in total biomass, root-to-shoot biomass ratios declined somewhat with increasing temperature for the two biotypes. The ratios obtained for monoecious *Hydrilla* on the inorganic sediment at 12° to 16° C were about twice those of the dioecious at the same temperatures. However, these high values are considered artifacts due to the sensitivity of this ratio to insignificant variations in biomass accrual under unfavorable growth conditions.

Shoot length and shoot number

Although total biomass production was similar for both biotypes. the method by which the biomass was allocated morphologically was distinctly different
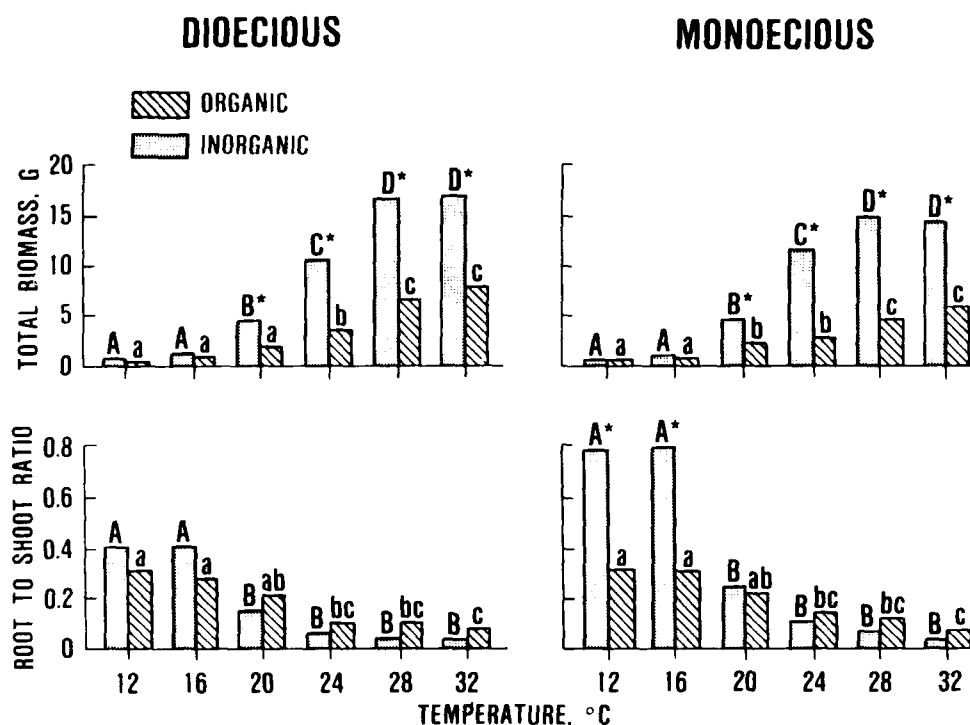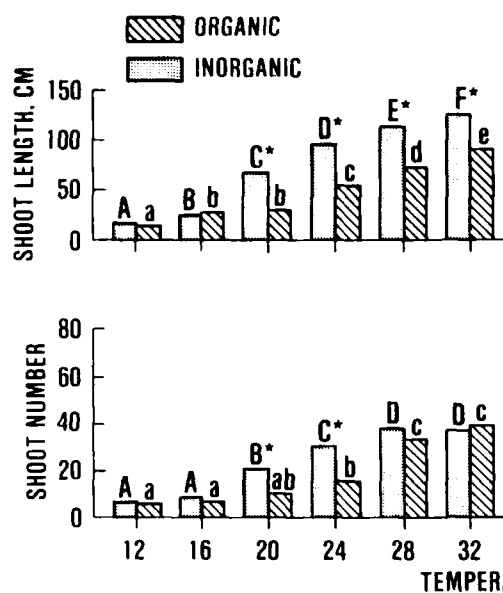
DIOECIOUS MONOECIOUS

Figure 1. Effects of temperature and sediment type on the growth of dioecious and monoecious *Hydrilla*. Within each subfigure, biomass values or root-to-shoot ratios sharing the same letter (uppercase for inorganic sediment and lowercase for organic sediment) do not differ significantly from each other. Asterisks denote significant effects of sediment type on growth. Duncan's Multiple Range Test was used to determine statistical significance at $P < 0.05$

(Figure 2). With increasing temperature, dioecious *Hydrilla* grew longer than the monoecious, especially on the inorganic sediment. Based on pooled means, the dioecious plants on the inorganic sediment increased in length ca. 25 percent over the monoecious variety. On the other hand, monoecious *Hydrilla* grew 'bushier' than dioecious *Hydrilla* on both sediments. Means compiled for each biotype indicate that the monoecious plants produced ca. 50 percent more shoots on the inorganic sediment and ca. 25 percent more shoots on the organic sediment than the dioecious plants. The increase in monoecious shoot densities on the more favorable inorganic sediment at temperatures between 16° and 24° C was striking. The ability of this biotype to produce high numbers of shoots may be an adaptation that increases reproductive potential through fragmentation, especially (as noted here) at moderate temperatures.

Tuber number and tuber mass

Graphs of tuber number and tuber mass are representative of monoecious *Hydrilla* only. Less than two tubers per replicate were formed by the dioecious biotype under any conditions, probably due to the short period of time during which the study was conducted (Haller, Miller, and Gerrard 1986; Van, Haller, and Gerrard 1978). The correlation between tuber number and tuber mass was rather high (r = 0.87). Again, the plants were more responsive to the inorganic sediment than to the organic sediment, with the influence of sediment type being
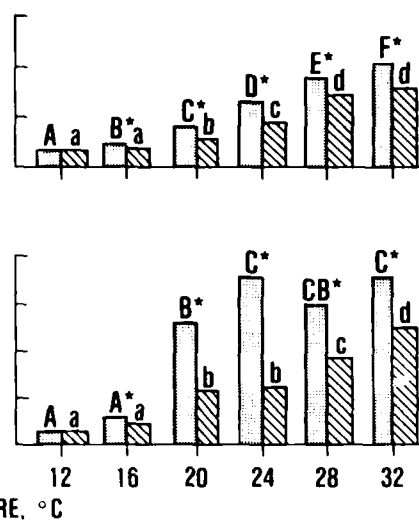
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Figure 2. Effects of temperature and sediment type on shoot morphology in dioecious and monoecious *Hydrilla*. Within each subfigure, shoot lengths or snoot numbers sharing the same letter (uppercase for inorganic sediment and lowercase for organic sediment) do not differ significantly from each other. Asterisks denote significant effects of sediment type on shoot morphology. Duncan's Multiple Range Test was used to determine statistical significance at $P < 0.05$
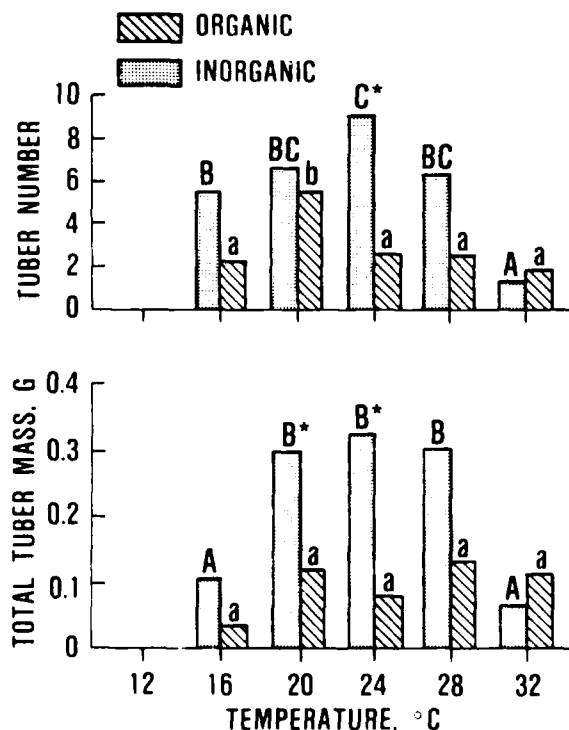

Figure 3. Effects of temperature and sediment type on tuber production in monoecious *Hydrilla*. Within each subfigure, tuber numbers and tuber mass values sharing the same letter (uppercase for inorganic sediment and lowercase for organic sediment) do not differ significantly from each other. Asterisks denote significant effects of sediment type on tuber production. Duncan's Multiple Range Test was used to determine statistical significance at $P < 0.05$

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greatest at 24° C. No tubers were produced at 12° C. In the inorganic sediment, maximum tuber production occurred at 24° C. Maximal tuber number in the organic sediment was reached at 20° C, although the mean mass was relatively low.

CONCLUSIONS

From these results, it is apparent that temperature and sediment type strongly affect the growth and morphology of monoecious and dioecious *Hydrilla*. In a general sense, the data are consistent with those of previous studies that have evaluated the independent influences of these factors on submersed macrophyte growth (Barko and Smart 1981a, b; 1983; 1986). In the present study, the interactive relationship of temperature and sediment on specific biotype responses has been elucidated. Specifically, biomass production was higher on the inorganic sediment at nearly all temperatures than on the organic sediment, and biomass values were highest on both sediments at highest temperatures. Although in nature growth of submersed macrophytes is dependent on numerous environmental parameters, water temperature and sediment composition determinations will be useful in assessing site-specific growth and distribution potential of these two *Hydrilla* biotypes.

Despite similarities in total biomass production, monoecious *Hydrilla* appears to possess adaptive capabilities beyond those of the dioecious biotype. The reproductive potential of monoecious *Hydrilla* is enhanced substantially by its ability to quickly form tubers and produce high shoot densities as a source of fragments. These functions together give it a competitive advantage in areas with relatively short growing seasons. The ability of monoecious *Hydrilla* to perform these functions at moderately low to intermediate temperatures may in part explain its present establishment in northern localities. Furthermore, its tuber and shoot production capabilities on organic sediments could extend its propagation to sites with sediments less tolerated by other submersed macrophyte species.

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Effects of Water Chemistry on Aquatic Plant Species: Growth Limitation by Inorganic Carbon

by
R. Michael Smart* and John W. Barko*

BACKGROUND

It has long been known that the chemical composition of water can affect the growth and distribution of submersed aquatic plants. Many water chemistry parameters have been correlated with the distribution of different species of aquatic plants. However, since many of these parameters are correlated with each other, and since water chemistry is often related to sediment composition, it is difficult to ascribe differences in growth and distribution to specific water chemistry parameters. This difficulty hinders our ability to predict the likelihood of excessive growth of submersed aquatic vegetation in particular water bodies.

OBJECTIVES AND APPROACH

The objective of this work unit is to determine the influence of specific water chemistry parameters on the growth and potential distribution of submersed aquatic plant species. The work unit was initiated in FY 82 and is scheduled for completion in FY 89. While much of the preceding research has been conducted in the laboratory, we will be verifying our results under field conditions during the next few years.

From our previous studies of plant growth in relation to water chemistry (Smart and Barko 1984a, b; 1985a, b; 1986) we have learned that the water chemistry parameter of primary concern is dissolved inorganic carbon (DIC). Under certain conditions dissolved calcium may affect plant growth and distribution, but we believe this to be a relatively rare occurrence (Smart and Barko 1984b, 1986). Similarly, dissolved potassium may also limit the growth of submersed aquatic plants (Barko 1982), but again, we expect this to be a relatively rare occurrence (Smart and Barko 1986).

Submersed aquatic plants exert such a high demand on DIC that it is difficult to conduct controlled studies on the growth responses of these plants to different levels of DIC (Smart and Barko 1985b, 1986). After only a few weeks' growth, DIC levels can be depleted to less than half of their original concentration (Smart and Barko 1986).

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Under field conditions, DIC removed by photosynthetic uptake is replenished by atmospheric exchange, sediment respiration, water column respiration, and advection. In our experimental tanks (described in Barko and Smart 1980), DIC is replenished mainly by atmospheric exchange. To minimize DIC depletion, we conduct most of our studies under relatively uncrowded conditions with only a few containers of plants growing in each tank. We also accelerate the exchange process by aerating the tanks with compressed air. We have also found that augmenting the CO_2 concentration in the aerating gas can help to maintain DIC levels (Smart and Barko 1985a, 1986). While these practices partially alleviate the problem of DIC depletion, they also minimize the likelihood of growth limitation by carbon and maximize the likelihood of limitation by sediment nutrients.

Experiment 1

The objectives of the first experiment reported here were to examine the ability of *Egeria densa*, *Hydrilla verticillata*, and *Potamogeton nodosus* to deplete DIC levels, and to determine the influence of different levels of DIC on the growth of these species.

The experimental design consisted of three levels of DIC: 3.5, 10.5, and 21 mg/ℓ. These are the same levels used in our earlier work with *Myriophyllum spicatum* (Smart and Barko 1986). Two levels of airstream CO_2 were employed: ambient and 4X ambient. DIC depletion was measured in solutions aerated with ambient air. Similar solutions aerated with air containing 4X ambient CO_2 concentrations were used for determining the influence of DIC on growth. We reasoned that the additional CO_2 would maintain DIC at initial levels, allowing for an evaluation of the effects of DIC on plant growth.

Experiment 2

Since both nitrogen and DIC appeared to limit species growth, we elected to conduct an additional experiment to examine possible interactions between these variables. This second experiment was conducted with *Egeria densa*, *Hydrilla verticillata*, and *Myriophyllum spicatum*. The experimental design included two airstream CO_2 concentrations: ambient and 10X ambient. The reason for employing the tenfold CO_2 level was that a fourfold increase had not prevented DIC depletion during the first experiment. Two sediment nitrogen treatments were employed: a control sediment and the same sediment after fertilization with NH_4Cl. This experiment was conducted at the midlevel of DIC (10.5 mg/ℓ).

RESULTS

Experiment 1

All species rapidly depleted DIC—particularly in the higher DIC solutions. Representative data for *Hydrilla* are shown in Figure 1. The period of rapid decline between week 2 and week 3 coincides with the period of active plant growth. Augmenting the airstream CO_2 concentration did not prevent the depletion of CO_2 from the high carbon solution. In effect, the levels of DIC in mid- and high-DIC
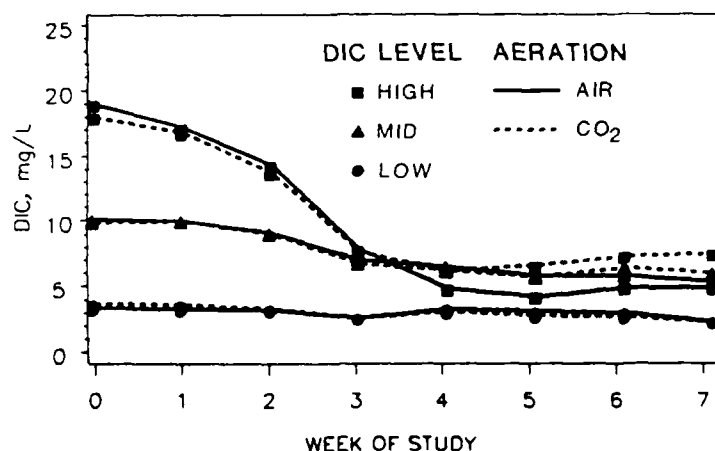
Figure 1. Changes in DIC in experimental solutions of different DIC levels during the growth of *Hydrilla verticillata* in the first experiment. The influence of a fourfold increase in CO_2 concentration of the aerating gas is shown by dashed lines

solutions were not significantly different throughout much of the study period. Thus, the second objective, to determine the influence of different levels of DIC on the growth of these species, could be only partially resolved.

Growth of all species was responsive to increasing DIC as exemplified by the growth response of *Egeria densa*, shown in Figure 2. These species did not generally respond to increased airstream CO_2 concentration. However, as previously shown, increasing the CO_2 concentration fourfold did not prevent the depletion of DIC; thus, the lack of a growth response to increasing CO_2 is not surprising. Examination of tissue nitrogen levels indicated that the growth of all three species was limited by nitrogen at the higher DIC levels. Thus, the growth responses in this study appear to have been influenced by both nitrogen availability and DIC.
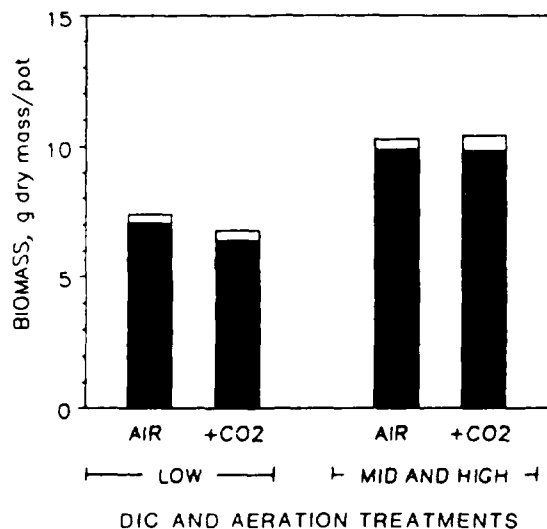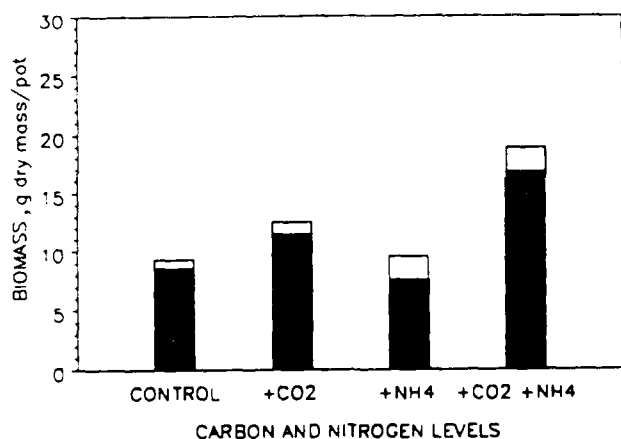

Figure 2. Biomass response of *Egeria densa* in relation to solution DIC and airstream CO_2 concentration in the first experiment
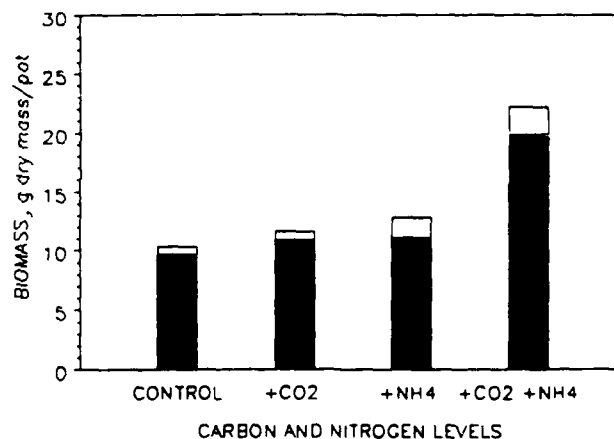
Experiment 2

Biomass production of *Egeria* responded to an increase in CO_2 but not to an increase in sediment nitrogen (Figure 3a). Growth of this species was thus considered to be carbon-limited. However, increasing the level of airstream CO_2 increased biomass production and resulted in subsequent nitrogen limitation in the high-CO_2 treatments. Maximum growth of *Egeria* was attained in the high-CO_2/high-nitrogen treatments.

Biomass production of *Hydrilla* did not respond to independent additions of either CO_2 or nitrogen alone (Figure 3b). However, the combined addition of both CO_2 and nitrogen promoted a dramatic increase in biomass production, indicating that biomass production in *Hydrilla* was limited by both carbon and nitrogen availability.

Biomass production of *Myriophyllum* responded to addition of nitrogen but not to addition of CO_2 (Figure 3c). Growth of this species was thus considered to be nitrogen-limited. However, increasing nitrogen availability increased biomass production and resulted in subsequent carbon-limitation in the fertilized treatments. Again maximum growth was attained in the high-CO_2/high-nitrogen treatments.

a. Egeria densa

b. Hydrilla verticillata
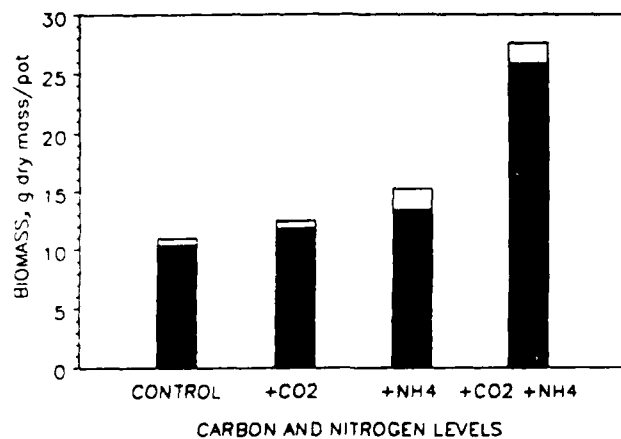
c. Myriophyllum spicatum

ıre 3. Biomass responses in relation to airstream concentration and sediment nitrogen level in the second experiment

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DISCUSSION

While the growth of all of these species was limited by nitrogen, limitation occurred only at very high levels of estimated areal biomass—on the order of 1,500 g dry shoot mass/m² sediment surface. These levels of biomass are rarely attained under field conditions. Carbon limitation, however, occurred at much lower levels of estimated areal biomass—ranging from 50 to 75 g/m² water surface. Thus, while nitrogen availability ultimately controls the level of biomass attainment, carbon availability controls the rate at which this biomass is accrued.

These results suggest that the growth of rooted, submersed aquatic plants in eutrophic systems may be limited by the availability of inorganic carbon. Given adequate temperature and irradiance regimes, the most likely limiting nutrients are carbon and nitrogen. While sediment nitrogen is potentially limiting, most fine-textured sediments contain adequate nitrogen to support nuisance levels of submersed plant biomass for many years. The extent of the aquatic weed problem may thus depend on the availability of inorganic carbon for photosynthesis.

Unfortunately, there does not seem to be a simple relationship between solution carbon level and biomass production or species distribution. The reason for this is that inorganic carbon availability is only indirectly related to water chemistry. While waters of higher alkalinity generally contain a greater quantity of readily available carbon, it is the rate of regeneration of this carbon that is of primary importance. Actively growing submersed macrophyte populations are capable of depleting the DIC available in the water column in a matter of days or weeks. Continued growth of these populations will depend on the carbon regenerating capacity of the system. The potential of a water body for supporting problem-level populations is thus more dependent on the recycling of carbon than on ambient water chemistry per se. While biomass production may be proportional to inorganic carbon supply, the supply of carbon is dependent on rates of water column respiration, sediment respiration, atmospheric exchange, and advection in addition to the chemical composition of the water.

The species examined here exhibited differences in their relative responsiveness to nitrogen and carbon. Under nitrogen-limiting conditions species with high affinities for nitrogen uptake may possess a competitive advantage. Conversely, under carbon-limiting conditions, species that can efficiently utilize bicarbonate may be favored. Species distribution as well as biomass production may thus be dependent on both nitrogen availability and inorganic carbon supply.

ONGOING AND FUTURE RESEARCH

In order to apply these laboratory results to field conditions, we plan to conduct both descriptive and manipulative experiments in waters of different chemistry compositions. We have begun to quantify the rates of replenishment of DIC from atmospheric, water column, and sedimentary sources. In addition, we plan to determine the relative importance of carbon and nitrogen limitation under field conditions.

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Waterhyacinth Phenology and Carbohydrate Allocation

by

Kien T. Luu*

INTRODUCTION

Research efforts to control waterhyacinth have focused on mechanical, chemical, and biological methods. Mechanical control is often infeasible and prohibitively expensive. In fact, mechanical methods of control involving maceration of the plants may actually promote vegetative reproduction. Though effective herbicidal controls are available, the publicly perceived impacts on the aquatic environment make them less desirable. Biological control agents (insects, pathogens, etc.) are being studied extensively, but biological control measures may also fail if the plants are reproducing and growing faster than the rate at which the control agent can feed and reproduce.

Perhaps a more logical method for managing waterhyacinth would be an approach involving one or more of the above methods implemented concurrently or sequentially. This approach requires an understanding of the phenological characteristics and energy (carbohydrate) allocation of waterhyacinth to determine critical growing periods. Once this is known, implementation of effective control methods can be reasonably expected.

There is little information about identifying carbohydrate reserves in waterhyacinth. Most studies that reported carbohydrate reserves in waterhyacinth focused on the total carbohydrate content and specifically on the potential use of waterhyacinth for methane gas production or for feed quality (Agrupis 1953, Russell 1973, Tucker 1981a, b, Tucker and DeBusk 1981). Carbohydrate reserves are essential to the survival and regrowth of a plant and to the production of plant tissues during periods when food utilization exceeds photosynthetic activity (Smith 1975). The cyclic pattern of use and storage of carbohydrate reserves is influenced by prevailing environmental conditions. Carbohydrate content of the whole plant may be a very good indicator of plant vigor, and carbohydrate reserve may be a good marker for the prediction of the postwinter regrowth capacity of a perennial plant.

The objectives of this study are to: (a) identify the important morphological and phenological characteristics of waterhyacinth, (b) determine seasonal carbohydrate distribution within plant structures, and (c) suggest management strategies based on phenology and/or carbohydrate reserves.

The following discussion summarizes some preliminary results and observations on the growth characteristics of waterhyacinth.

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MATERIALS AND METHODS

An outdoor experiment was initiated at WES in August 1986 to evaluate the growth pattern of waterhyacinth and to determine the seasonal trends of carbohydrate distribution among plant parts. Waterhyacinth plants were grown in a holding tank for several months. Young ramets of similar size (four leaves) were selected and cultured outdoors in six 1,300-*l*, epoxy-coated, wooden tanks (26 × 76 × 76 cm). A nutrient medium of 10-percent Hoagland solution was used (Hoagland and Arnon 1950). Water temperature was maintained at 27° to 30° C by recirculating water through temperature-regulating chillers (Remcor Products Co., Chicago, Ill.). Daily maximum and minimum temperatures of air and water were recorded by self-registering thermometers.

Two ramets were placed in each tank to closely observe the branching pattern. After a month, two ramets of the second- and third-generation offsprings from the original mother plants were selected and observed twice weekly for leaf growth and leaf longevity. The total number of plants per tank was recorded weekly until plant density reached a maximum. During the hot months (July, August, and September), the plants were shaded from 60 percent of available ambient radiation using shade cloth, to prevent the water surface from reaching temperatures greater than 34° C, and thereby preventing detrimental stress to the growing plants. A randomized complete block experimental design with two blocks and three replications was used. Measured parameters included leaf size, number of leaves, leaf longevity, ramets/plant, plant density, stolon length, inflorescence size, inflorescences/tank, and carbohydrate content. Different plant parts (rhizome, root, young, mature, and old leaves) were sampled biweekly or monthly and were dried in a forced-air oven at 58° C for 48 hr to a constant dry weight for carbohydrate analyses.

INITIAL OBSERVATIONS ON GROWTH CHARACTERISTICS

Two distinct types of waterhyacinth flowers were observed based on the style size: long and midstyle. On most flowers the androecium consists of three short stamens and three long stamens. On a midstyled flower, the stigma is intermediately positioned between two groups of anthers, whereas on a long-styled flower, the style is longer than both groups of stamens, and the stigma is at a higher position than the two groups of anthers. There were only 18 long-styled inflorescences among 297 observed. We intend to verify any genetic makeup differences between plants with different flowers.

Factors that control the onset of flowering in waterhyacinth are not well understood. Richards (1980, 1982) demonstrated that nutrient deficiency stimulated flowering, while Watson (1984) pointed out that increased nutrient concentration or exogenously supplied gibberellic acid may also stimulate flowering. Pieterse, Aris, and Butter (1976) reported that gibberellic acid induced profuse flowering in waterhyacinth. Bock (1966) surveyed many herbarium specimens from the tropics and southern Florida and cited that waterhyacinth may bloom year round. This indicates that photoperiod may not be a stimulant

of flowering. However, in our experiment, flowering occurred in only one block of three tanks which daily received about 1 to 2 hr more sunlight than the other block. It is thus evident that photoperiod was indeed involved in flowering of waterhyacinth. Photoperiod may not be the only factor influencing flowering, since endogenous plant hormones probably play a role.

PRELIMINARY RESULTS ON GROWTH CHARACTERISTICS

During a 10-week observation period, plant density exponentially increased during the first 8 weeks. By the ninth week, crowded conditions prevailed and plant density leveled off slightly. Average plant density of one plant/m^2 multiplied to 142 plants/m^2 in only 10 weeks under our experimental conditions.

Synchronization of ramet development with leaf emergence did not occur. Speed and number of ramets developed was directly related to the available interplant space and the nutrient status of the growing medium. A single plant produced a maximum of six ramets with an average of five ramets during the 10-week growing period. Richards (1982) found an average of seven ramets per plant after 6 weeks of growth in full-strength Hoagland solution.

In waterhyacinth, the stolon length may indicate the density of the plant stand. In our experiment, the average length of a fully developed stolon was 26 cm (ranging from 17 to 36 cm) with a growth rate of 1 cm/day. Penfound and Earle (1948) have reported that in a closed stand of waterhyacinth, the stolons averaged about 5 cm in length, but in an open stand, they may reach a length of 46 cm. The average stolon length indicated that the waterhyacinth stand in our experiment was not very dense. However, another factor that may have contributed to the differences in average stolon length in our results, as compared to those of Penfound and Earle, is sampling method. We measured only the fully developed stolons, while Penfound and Earle may have included some immature stolons in their samples.

Leaf measurements were made every other day. This summary presents only the data from the first 10 leaves. The first leaf lived up to 24 days. The longevity of the second through the tenth leaf ranged from 38 to 44 days. It took less than a week for the first two leaves to reach maturity; the subsequent leaves required 10 days to two weeks for full development. Center and Spencer (1981) have reported that a waterhyacinth plant produced a new leaf every 5 to 10 days, and the leaves lived for 35 to 50 days.

PROSPECTIVE USEFULNESS OF THE RESULTS

The results of this study may be used to: 1) indicate plant vigor and predict regrowth capacity; 2) reveal the feeding preferences of selected biological control agents; 3) establish plant growth cycle; and, 4) identify weak periods during the plant growth cycle, which make it susceptible to various control techniques.

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Field Studies of Submersed Aquatic Vegetation in the Potomac River

by
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ABSTRACT

Submersed aquatic vegetation was found in the freshwater tidal Potomac River in 1983 for the first time in decades. The vegetation colonized only the upper tidal Potomac River, a 24-km reach from Washington, D.C., to Marshall Hall, Maryland, in 1983-84, but, by 1986, plants had spread 13 km further downstream into the lower tidal Potomac River (Marshall Hall, Maryland, to Quantico, Virginia). Several water-quality changes resulting from improvements at Blue Plains Sew age Treatment Plant preceded the return of plants to the tidal river. Tertiary se· age treatment and advanced wastewater treatment (nitrification) resulted in a decrease in phosphorus loading, a change in nitrogen loading from NH_4 to NO_3, an·l a decrease in suspended solids. Water clarity improved significantly to a mean Secchi depth of 85.5 cm in the upper tidal river in 1983 as compared with a mean depth of 51.8 cm in 1978-81. Water clarity has remained high (~85 cm) through 1986. Because nutrient loading declined and water clarity increased simultane ously with the return of the submersed macrophytes in the tidal Potomac River, it is not possible to attribute the resurgence of vegetation conclusively to either factor.

The lower tidal river continues to be nearly devoid of plants in comparison to the upper tidal reach and the well-vegetated transition zone of the estuary further downstream (Quantico, Virginia, to Morgantown, Maryland). Reduction of photosynthetically active radiation due to increased phytoplankton populations downstream may be responsible for the observed lack of vegetation in the lower tidal river. During the growing seasons of 1985 and 1986, suspended sediment, chlorophyll a, and light penetration at different wavelengths were measured in the upper tidal river, lower tidal river, and transition zone. Chlorophyll a concentrations were generally highest in the lower tidal river, whereas suspended sediment concentrations were very similar in each reach. Extinction coefficients at 430 nm (blue) and 665 nm (red) were significantly correlated with chlorophyll a concentration; the extinction coefficients were highest and the depth of the photic zone was least in the lower tidal river.

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Hydrilla verticillata, Vallisneria americana, Myriophyllum spicatum, Heteranthera dubia, Ceratophyllum demersum, and *Najas guadalupensis* were the most abundant species of submersed aquatics found in surveys during 1983-84. In 1984, *Hydrilla* comprised approximately 25 percent of the plant biomass, except in dense *Hydrilla* beds near Dyke Marsh. A 3-year field study on competition between *Hydrilla* and other species showed *Hydrilla* cover had increased from about 5 percent to nearly 100 percent cover in test grids by 1986. *Hydrilla* now dominates about 75 percent of the vegetated areas in the upper tidal river.

A water quality study in 1986 showed that water velocity, chlorophyll *a*, and suspended-sediment concentrations were lower inside than outside dense plant beds at low, mean, and high tide. Diurnal water quality measurements showed that dissolved oxygen, temperature, and pH inside the beds were consistently higher during the afternoon and lower at dawn.